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THE NATURE OF THE INHIBITING SUBSTANCE EMITTED BY GERMINATING UREDIOSPORES OF *PUCCINIA GRAMINIS* VAR. *TRITICI*[1]

BY F. R. FORSYTH[2]

Abstract

The self inhibition of germination of urediospores of *Puccinia graminis* Pers. var. *tritici* Erikss. & Henn. has been consistently demonstrated in Warburg flasks. Four ways of overcoming the effect of the inhibitory substance have been discovered: use of a sufficiently large volume of water in relation to weight of spores; the placing of a silver nitrate solution in the center well; and the introduction of acetone or ammonia vapor into the flask atmosphere. The vapor of trimethylethylene inhibits germination of the urediospores in the same way that the natural inhibitor does, and the same means can be used to counteract its effects. The similarity of the absorption spectra of the inhibitor and of trimethylethylene in acetone solutions indicates that trimethylethylene is the natural inhibitor. Cyclohexene has formative and inhibitory effects on the germ tubes and these effects can be overcome by vapor of acetone.

Introduction

At the Plant Pathology Laboratory, Winnipeg, a study has been made of the germination and subsequent growth and respiration of urediospores of wheat stem rust, *P. graminis* var. *tritici*, in Warburg flasks. Early in this study it was found that viable urediospores would not germinate in the closed flasks at 11°, 15°, and 22° C., but that a control consisting of similar spores in an open container showed a high percentage of germination.

Dickson (5) had reported the observation that germinating rust spores produce a volatile substance which, under certain conditions, inhibits germ tube elongation. Experiments were therefore undertaken to discover the nature of the inhibitor.

Materials and Methods

A refrigerated Warburg water bath and a standard manometric apparatus were used in the manometric studies. The temperature of 11° C., which allowed excellent urediospore germination while it limited the rate of increase of bacteria, was used in all of the respiration and germination studies.

[1] *Manuscript received March 14, 1955.*
Contribution No. 1442 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] *Plant Physiologist, Plant Pathology Laboratory, Winnipeg, Manitoba.*

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Urediospores of several races of wheat stem rust were used in the tests. In the first experiments the spore source and age were variable. These early tests demonstrated the variability to be found in the respiration rate and the germination percentage of spore samples of different ages, hosts, or storage history. Consequently in later experiments the spores (race 56) were produced on seedlings of Little Club wheat (*Triticum compactum*) in growth chambers under artificial lighting with controlled air flow, temperature, and humidity. The use of spores harvested regularly from plants grown under controlled conditions seemed to offer a safeguard against inconsistent results.

The spectrophotometric analyses were carried out by the use of a Hilger Uvispek spectrophotometer located in the Botany department of the University of Manitoba.

Procedure and Experimental Results

Natural Inhibition of Spore Germination and the Nullification of Inhibition

Experiments which had been designed to study the respiration rate of germinating urediospores proved to be more suitable for demonstrating the presence of an inhibitor of germination. Consequently a standard experimental procedure for demonstrating the presence of a germination inhibitor was adopted. Two-milligram samples of urediospores for each of five replicates in the Warburg flasks and one for an open flask test were prepared. A sample complement of reactants in a flask consisted of 2 mgm. of urediospores, 2.8 ml. boiled-out distilled water, and 0.2 ml. 20% potassium hydroxide to absorb carbon dioxide. At approximately the same time that the flasks were connected with the manometers and placed in the water bath, an Erlenmeyer flask with distilled water and urediospores was suspended unsealed in the water bath. The hourly and total uptakes of oxygen by the spores from the flask atmospheres were recorded in most experiments.

Whenever an experiment had been completed, the spores were poured out into a Petri dish and a count was made of the percentage of spores that had germinated. This counting was difficult because of the intertwining of hyphae with non-germinated spores and the tendency for unilayered "rafts" of spores of high germination percentage to roll up into a mass which could not be properly examined under the microscope. Thus the percentages recorded are only approximate, and, accordingly, no significance is attached to small apparent differences.

The reality of the inhibition was established in 10 experiments: four with spores of wheat stem rust race 15B, five with wheat stem rust spores collected in the field, and one with spores of crown rust, *Puccinia coronata* Corda var. *avenae* Erikss. & Henn.

Table I is a summary of the pertinent results from the 10 experiments. The reduction in the germination percentage was obvious before counting. Spore lots of high germination showed a white appearance due to masses of germ tubes. Those of low germination showed the brown color of the ungerminated spores. The total oxygen uptake during three and one-half hours

TABLE I

GERMINATION AND RESPIRATION OF UREDIOSPORES CONFINED IN WARBURG FLASKS AND COMPARATIVE GERMINATION IN OPEN FLASKS

Expt. No.	Race of rust	Wt. of spores added, mgm.	Approximate germination % in Warburg flask*	Approximate germination % in open flask**	Total O_2 uptake in 3½ hr. in Warburg flasks, µl.
1	*P. coronata*	2	5	90	35.05
2	Unknown races of *P. graminis* var. *tritici*	2	50	90	22.63
3	"	2	Trace	95	31.64
4	"	2	7	90	32.49
5	"	2	4	90	—
6	"	2	6	90	28.92
7	15B	2	10	90	23.02
8	"	2	5	95	25.80
9	"	2	Trace	95	21.81
10	"	2	10	90	25.34

** Flask contents: 2 mgm. urediospores of above races; 2.8 ml. distilled water; 0.2 ml. 20% KOH. Temperature was 11° C.*

*** The open flask did not contain potassium hydroxide. Preliminary experiments had shown that the presence of potassium hydroxide in the center well of the Warburg flask is not a factor in the prevention of spore germination.*

was, of course, variable because of the varying percentages of germinating spores. Thus whenever 2 mgm. of viable urediospores were placed on 2 ml. or less of distilled water in a Warburg flask an inhibition of germination occurred which was as much as 99% effective.

Four ways of overcoming this inhibition were found:

(1) By increasing the water supply to the spores in relation to the weight of spores used.
(2) By the use of 20% silver nitrate solution in the center well of the flask.
(3) By the addition of approximately 1.4% acetone and water solution in the center well.
(4) By the placing of approximately 0.06 *M* ammonia solution in the center well of the flask.

Since hydrogen cyanide (HCN) is produced by certain basidiomycetes (9, 14) there was the possibility that it was produced by germinating urediospores of stem rust and that it could be the natural inhibitor. It is known that HCN distills into a solution of silver nitrate and thus silver nitrate might be expected to absorb the HCN from the flask atmosphere and to overcome the inhibition. This reasoning prompted the use of the silver nitrate in the center wells. However, as was subsequently discovered, the silver nitrate did not act in this instance through an absorption of HCN.

To test further the hypothesis that the inhibiting substance was HCN, ammonia was added to the silver nitrate in the flask. In the presence of ammonia any HCN that might be formed would not be precipitated as silver cyanide. Instead, the complex ions $Ag(NH_3)_2^+$ and $Ag(CN)_2^-$ would form, removing the Ag^+ from solution and redissolving any AgCN which precipitated.

At this time it was hoped that an absorption spectrum of the inhibitory substance could be found by spectrophotometric analysis of the diluted silver nitrate from the center well.

Although the addition of ammonia to the silver nitrate did obviate the inhibition it was soon discovered that ammonia by itself had a similar effect. The effect of ammonia alone cast doubt on the hypothesis of cyanide production by the urediospores.

Before any absorption spectrum was obtained the evidence indicated that acetylene could be causing the inhibition. Silver nitrate should form silver acetylide with acetylene and this reaction should be aided by the presence of ammonium hydroxide (13). Furthermore, it was because of the known solubility of acetylene in acetone that the latter, which proved so effective in overcoming the inhibitor, was used in the center wells. Also it was the use of acetone as an absorbent that enabled an absorption spectrum of absorbed materials to be determined in the ultraviolet region and that led finally to the identification of trimethylethylene as the inhibitor.

The action of water and silver nitrate in neutralizing the inhibitory substance was tested by using various proportions of water to a constant weight of spores and by using a 20% silver nitrate solution in the center well. A folded filter paper was inserted in the center well to increase the effective surface. Similar tests were made with a stock solution of acetone at a concentration of 14.28% and one of ammonia at 0.66 *M*. At these concentrations acetone and ammonia prevented urediospore germination but at lower concentrations germination took place.

Fig. 1 indicates that the total volume of oxygen taken up in 18 hr. by 2 mgm. of spores increases as the amount of water in the flask is increased.

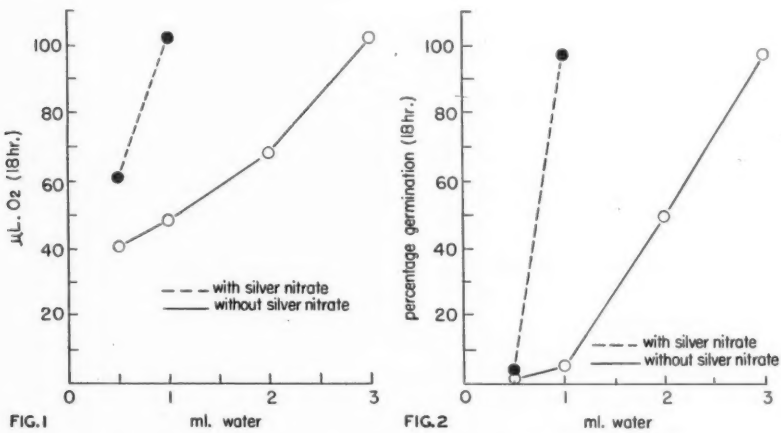

FIG. 1. Effect of water and of silver nitrate in overcoming the inhibition.
FIG. 2. Effect of water and of silver nitrate in overcoming inhibition of germination.

TABLE II

THE EFFECT OF THREE CONCENTRATIONS OF ACETONE AND AMMONIA ON SPORE GERMINATION

Treatment	Concentration in flasks*	Approximate germination, %
Control	—	Zero
Stock acetone	14.28%	Zero
1/10 stock	1.43%	50
1/100 "	0.14%	80
Stock ammonia	0.66 *M*	Zero
1/10 stock	0.07 *M*	95
1/100 "	0.007 *M*	90

* *Flask contents: 2 mgm. urediospores of P. graminis var. tritici race 15B-3; 1.0 distilled water; 0.4 ml. ammonia or acetone solution as indicated, in the center well; 0.2 ml. of 20% KOH in sidearm. Temperature 11° C.*

Also (See Fig. 2) the increase in oxygen uptake was paralleled by an increase in the germination percentage, from a trace with 0.5 ml. of water to approximately 98 per cent with 3 ml. per 2 mgm. of spores.

Regarding the use of silver nitrate, Fig. 1 indicates the increase in oxygen uptake whenever silver nitrate is present, and Fig. 2 illustrates the increase in percentage of germination when the water supply would otherwise limit the number of spores able to germinate.

The third and fourth means of overcoming the effect of the natural inhibitor are shown in Table II. Either 1.43% by weight of acetone or 0.07 *M* ammonia in the center well of the flasks consistently overcame the inhibition. The silver nitrate at times failed to prevent low germination and it was not used in later experiments.

Ultraviolet Absorption Spectrum of the Inhibitor

The fact that an acetone solution which was not in direct contact with the germinating spores would overcome the effect of the inhibitor suggested that a volatile substance had been absorbed from the flask atmosphere by the acetone.

A 3-ml. sample for spectrophotometric analysis was obtained by combining the 0.4 ml. samples of 1.4% acetone solutions from the center wells of seven Warburg flasks which had spores germinated on a water surface. It was necessary to dilute the acetone solutions by about one-third to obtain the required volume of sample. By means of a spectrophotometer and the use of a 1-cm. quartz cuvette the absorption spectrum of the acetone plus the inhibitor was obtained from 2000 to 3000 Ångstrom units (Å). The acetone solution alone was found to have an absorption peak at 2660 Å. On the assumption that the acetone alone was responsible for this absorption peak, a comparable acetone solution was prepared and its absorption spectrum determined. Since the acetone solution alone absorbed much less light in

the ultraviolet region from 2000 to 2300 Å than did the combined solution (acetone plus inhibitor), the absorption due to the comparable acetone solution was subtracted from the combined one to give that attributable to the inhibitor alone.

A stronger solution of the inhibitor in acetone was obtained by germinating 150 mgm. of urediospores, race 56, on the surface of distilled water in a 20-liter pyrex carboy. The inhibitor was absorbed into 25 ml. of 0.5% acetone in a 50-ml. beaker floating among the spores for a 21-hr. period. By the method of difference outlined above a spectrum for the inhibitor was obtained. The resulting spectrum resembled the published spectrum of 2-methylbutene-2 (2, 9, 14); and the probable chemical reactions with silver nitrate, acetone, and ammonia (7) suggested so strongly the presence of an unsaturated bond in the volatile substance that a research grade sample of 2-methylbutene-2 (trimethylethylene) was obtained from Phillips Petroleum Company of Bartlesville, Oklahoma, for comparison with the natural inhibitor. By trial a solution was prepared, containing one drop of trimethylethylene in 50 ml.

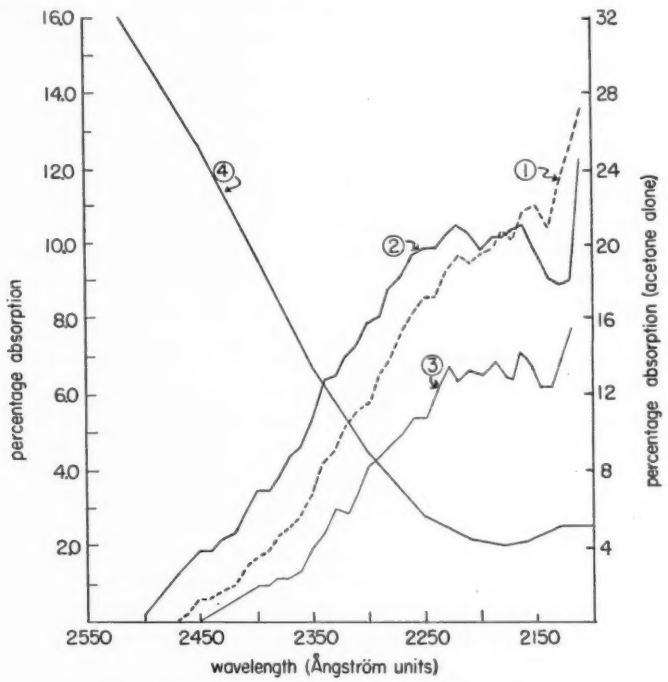

FIG. 3. (1) Absorption spectrum of trimethylethylene (by difference).
(2) Absorption spectrum of natural inhibitor (by difference).
(3) Absorption spectrum of trimethylethylene after vaporization and recovery in acetone.
(4) Absorption spectrum of acetone solution used in obtaining (1) and (2).

of comparable acetone solution, which was roughly equivalent in concentration to that of the natural inhibitor. Trimethylethylene was vaporized in a large carboy and then absorbed by a weak acetone solution by a method similar to that used for collecting the natural inhibitor. An absorption spectrum was then obtained of this sample by the method of difference.

Fig. 3 shows the absorption spectra of (1) the natural inhibitor, (2) trimethylethylene obtained by the method of difference, (3) trimethylethylene after vaporization and collection in acetone, and (4) a comparable acetone solution as used in determining (1) and (2). The similarities in form of the ultraviolet absorption spectra of trimethylethylene and the natural inhibitor are apparent, and with the presence of the known peak at 2150-2170 Å (2) the evidence is virtually conclusive that the natural inhibitor is trimethylethylene.

Trimethylethylene as an Inhibitor of Spore Germination

Proof was required that trimethylethylene acted biologically like the natural inhibitor. A series of solutions with trimethylethylene concentrations of 0.1 to 1650 p.p.m. by weight was prepared by shaking up the required volume with distilled water. Two-milligram samples of urediospores were germinated on the liquid surface in Warburg flasks (see Table III). When it was required, trimethylethylene in vapor form was prepared in a suction filtering flask by adding a drop of the liquid to the flask at room temperature. The trimethylethylene vaporized rapidly and measured volumes of the vapor were passed through the Warburg flasks after they were sealed onto the manometers. This was accomplished by water displacement of the vapor so

TABLE III

INHIBITORY EFFECTS OF TRIMETHYLETHYLENE AND THEIR NULLIFICATION

Treatment and trimethylethylene conc.	Effect on germination			
	Acetone present 1.43 or 2.86%	Acetone absent	Ammonia present, 0.28 *M*	Ammonia absent
0.1 p.p.m. w/v water	Inhibitory effect nil	Inhibitory effect nil	Inhibitory effect nil	Inhibitory effect nil
1.1 " "	"	"	"	"
11 " "	"	"	"	"
110 " "	"	"	"	"
330 " "	"	"	"	"
660 " "	"	"	"	"
990 " "	"	"	"	"
1320 " "	"	"	"	"
1650 " "	"	"	"	"
100 p.p.m. v/v in vapor	50% germination	50% germination	"	"
200 " "	Trace "	Trace "	50% + germination	Trace
400 " "	Trace "	Trace "	Trace "	Trace

Flask contents: 1 mgm. of urediospores of P. graminis var. tritici race 56; 0.4 ml. of acetone or ammonia solution; 0.2 ml. 20% KOH; 2.4 or 2.8 ml. of distilled water to make a total volume of materials added of 3.0 ml. Temperature was 11° C.

that it was forced into the venting plug of the flask and out of the stopcock of the manometer. After 125 ml. of vapor had been flushed through, the venting plug was closed and the manometers and flasks were attached to the Warburg apparatus.

The results of the experiments to demonstrate the effects of trimethylethylene and to nullify them are tabulated in Table III. It is evident that a high concentration is required to inhibit the germination of spores if the trimethylethylene is shaken up with water. This result could be due to the low temperature (11° C.) and the consequent low volatility. The effect of the vapor form proved to be more easily demonstrated. The 100 p.p.m. v/v vapor of trimethylethylene did not inhibit. The 200 p.p.m. v/v vapor inhibited germination entirely and could not be overcome by acetone, probably because the acetone concentration required was itself inhibitory to spore germination. However, the ammonia at 0.28 *M* was able to nullify the effect of the trimethylethylene (see Table III) at 200 p.p.m. and was unable to do so at 400 p.p.m. Thus the similarities in inhibition and nullification are proved for the natural inhibitor and trimethylethylene.

An interesting point of difference between the urediospores of stem rust and leaf rust (*P. triticina* Erikss.) was revealed by the effect of trimethylethylene and acetone as inhibitors. The few spores that germinated when all others were inhibited by trimethylethylene and acetone were invariably found to be contaminant leaf rust spores.

Cyclohexene Compared with Trimethylethylene as an Inhibitor

While the hypothesis that trimethylethylene might be the natural inhibitor was being tested, it was considered worthwhile to test certain other chemicals that contained the double bond such as cyclohexene and isopropylethylene. The absorption spectra of cyclohexene or isopropylethylene could not be mistaken for that of the inhibitor. However, when cyclohexene was tested as an inhibitor of germination some interesting effects were revealed.

The cyclohexene solutions were prepared by shaking up with distilled water measured volumes, and thus calculable weights, of cyclohexene. The 2-mgm. samples of urediospores were germinated on the surface of the cyclohexene solutions in Warburg flasks. Records of the percentage germination, respiration, and form of the germ tubes were kept. Tests of the efficacy of the acetone vapors in overcoming the inhibition were performed in the same manner as were those with the natural inhibitor and trimethylethylene.

Cyclohexene was found to inhibit urediospore germination at concentrations of 400 p.p.m. At the lower concentrations of 200 and 100 p.p.m. the spores germinated but the germ tubes were approximately 100 μ in length, compared to a normal length of about 800 μ. The formation of bulbous ends on the tubes at the 200 and 100 p.p.m. level (see Figs. 6 and 5) was of great interest in that it raised the possibility that cyclohexene could be a factor, perhaps a hormone, for formative effect in *P. graminis* var. *tritici*.

The effect of cyclohexene on spore respiration is shown in Table IV in which the percentage uptake in μl. of oxygen is expressed as a percentage of

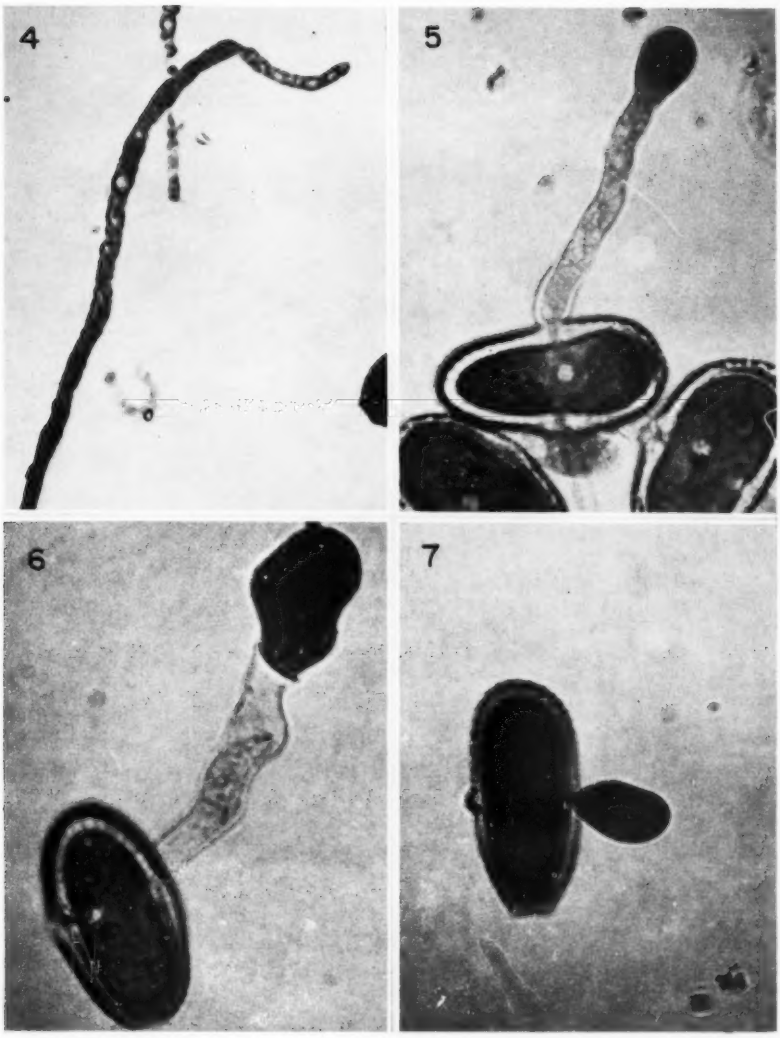

FIG. 4. Form of the normal germ tube of *P. graminis* var. *tritici* on water surface. FIGS. 5, 6, 7. Form of germ tubes on 100, 200, and 400 p.p.m. respectively of cyclohexene and water w/v. Magnification ×1000.

TABLE IV

INHIBITORY EFFECTS OF CYCLOHEXENE AND THEIR NULLIFICATION

Cyclohexene conc.	Effect on germ tube development	Effect of 1.4% acetone in center well	Respiration as % of control without acetone. µl. O_2
50 p.p.m. w/v	None	None	100+
100 " "	Bulbous end*, high % germination	High % germination	—
200 " "	"	"	76
400 " "	No germination		14

* *Fig. 4.*

Flask contents: 2 mgm. urediospores of P. graminis var. tritici race 56; 2.0 ml. cyclohexene sol'n.; 0.4 ml. 1.4% acetone when used; 0.2 ml. 20% KOH. Temperature 11° C.

the uptake of the control. Whenever the germination is inhibited by the 400 p.p.m. of cyclohexene there is, of course, a reduced respiration rate. However, in the case of the lower concentration of 200 p.p.m., which allows a high percentage germination, the respiration rate does not equal that of the control, and at the concentration of 50 p.p.m. there is consistently a greater oxygen uptake than in the control.

The nullification of the inhibition due to cyclohexene is effected for the 400 p.p.m. level by 1.4% acetone in the center well (see Table IV). Thus a similarity in means of overcoming the effect of cyclohexene and trimethylethylene is demonstrated. There is, however, the very distinct difference that cyclohexene has a formative effect on the germ tubes and trimethylethylene has not.

Discussion

Ethylene is given off by ripening fruits (11, 12) and by more than 16 different species of higher plants (4). The plant organs producing ethylene include petals, anthers, pistils, immature fruits, leaves, and tubers. The ethylene gas produced by the above-mentioned plant parts apparently depresses or increases their respiration rate depending upon the concentration in the air surrounding the plant and upon the time involved and the plant organ used. Roberts (15) showed that it is possible to inhibit the growth in length of roots and shoots of wheat without altering their carbon dioxide output.

The experiments described in the present work show that the germinating urediospores of *P. graminis* var. *tritici* produce trimethylethylene which prevents germ tube elongation. As far as the author is aware this is the first record of the production of this chemical by any plant part.

The purpose of this investigation was to gain information of possible value in explaining the nature of rust resistance and rust damage.* It is felt that

* *Since this paper was written the interesting work of C. E. Yarwood (19) has come to our attention. It is possible that the toxic gas which may cause acquired immunity to rusts is identical with the inhibiting substance produced by germinating urediospores of P. graminis var. tritici.*

since the rust organism produces such a toxic substance this knowledge may be used in the designing of experiments to explain the toxic reactions to be noted in certain wheat leaves consequent upon infection with stem rust. Although Denny (3) was unable to show any epinastic effects of trimethylethylene on potato shoots, *Solanum tuberosum* L., nevertheless compounds having the double bond such as hexene-1 are known to cause plant injury to the pinto bean, *Phaseolus vulgaris* L. (8). If it can be shown that trimethylethylene causes an acceleration of chlorophyll destruction in wheat leaves, as has been shown (1) for the action of emanations from *Penicillium digitatum* on green fruit, there would be good reason to believe that the chlorotic areas surrounding early infections of stem rust on wheat leaves are caused by chlorophyll destruction due to trimethylethylene.

To cause injury to the host by means of trimethylethylene the rust hyphae would merely need to grow into a medium of cells from which the trimethylethylene produced by rust metabolism could not escape. Substances of similar chemical constitution are, however, known to increase cell permeability (6) and it seems likely that trimethylethylene may produce this effect. According to Thatcher (17), high permeability is associated with susceptibility to stem rust. The observation by Suchorukov (16) that immunity to rust is caused by heightened ammonia content is explainable if it is assumed that trimethylethylene tends to increase host susceptibility because it increases cell permeability. Then, the nullification of its effects by ammonia would tend to increase resistance.

The significance of the formative effects of cyclohexene on the germ tube of *P. graminis* var. *tritici* and the similarity in inhibition and de-inhibition of this compound and trimethylethylene is obscure.

Acknowledgments

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SEEDLING BLIGHT, A FUSARIAL DISEASE OF ASPARAGUS[1]

By K. M. Graham[2]

Abstract

Incidence of seedling blight in Ontario can be correlated with proximity of mature plantations or with the presence of debris from a previous asparagus crop. As the disease was detected on seedlings grown in soil from "volunteer" plants growing in isolated habitats, the pathogen is considered to be indigenous. The pathogen penetrates directly into the embryonic region of the root tip, or through stomata on the hypocotyl, and colonizes the host both intercellularly and intracellularly. Its limitation to the cortex is regarded as unusual among the parasitic fusaria, which are mainly vascular. The fungus was identified as *Fusarium oxysporum* sensu Snyder & Hansen var. *redolens* (Wr.) Gordon. In comparison with *F. moniliforme* Sheldon, found to cause a root tip necrosis of asparagus seedlings, the cortical *Fusarium* produced less growth on basal medium plus cellulose but more growth on sucrose, and showed a greater ability to hydrolyze starch. Differing nutrient preferences are suggested as one explanation for the localized types of colonization exhibited by these two fusaria. The optimum soil temperature for disease incidence was established at 25°-30° C. The effect of temperatures above 30° C. is considered to be twofold: (1) inhibiting growth of the pathogen and (2) permitting the host to escape by inducing its more rapid emergence. Below 20° C., the pre-emergence phase of the disease is favored by a retardation of the rate of emergence. Both low and high levels of soil moisture retard emergence and favor pre-emergence blight. Under greenhouse conditions, incidence of the disease usually increased more rapidly when leguminous rather than nonleguminous residues were present in the soil and there was some evidence that the saprophytic growth of the pathogen was more profuse on certain nonliving substrates.

Introduction

Seedling blight of asparagus was unknown in Canada until the spring of 1945. At that time, a number of plantations in the vicinity of St. Catharines and Vineland, Ont., were affected so severely that they were destroyed and the land was used for another purpose. No comparable disease could be found in mature plantations, although growers had complained during recent years of inexplicable reductions in yields from fields which should have been in their prime.

A survey of the pertinent literature has revealed the occurrence of root rots of asparagus in Europe and America. Weise (22) in 1939 published an account of a root disease in Germany. He attributed this disorder to *Fusarium culmorum* (W. G. Sm.) Sacc. and suggested after extensive experimentation that control could be achieved by removing the "mound" from the crowns after harvest, in order to alter the conditions of temperature and moisture under which the pathogen thrived. Root rot has been reported from the Netherlands (20) and England (17).

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Joint contribution from the Department of Botany, University of Toronto, Toronto, Ontario, and (Contribution No. 1453) from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario. Based on a thesis presented in April, 1952, to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] *Formerly Demonstrator, Department of Botany, University of Toronto, and Assistant Plant Pathologist, Plant Pathology Laboratory, St. Catharines, Ontario; now Associate Plant Pathologist, Botany and Plant Pathology Laboratory, Ottawa, Ontario.*

Similar reports from the United States are not lacking. A destructive wilt and root rot of asparagus has been noted in New Jersey (21), Pennsylvania (13), Massachusetts (5), Oregon (6), Idaho (4), North Carolina (2), South Carolina (3), Illinois (1), Missouri (18), and Washington (8). Two investigators, M. T. Cook (9) and Sylvan I. Cohen (7), made persistent attempts to isolate the pathogen or pathogens involved. Cohen attributed the disorder to an organism which he named *Fusarium oxysporum* Schlecht. sensu Snyder & Hansen var. *asparagi* and considered it to be distinct from the German disease on the basis of its apparent preferential invasion of the vascular system. However, a careful translation of Weise's (22) paper reveals the statement: "They prefer the lumen of the water-conducting organs and grow therein over long distances. If one splits the asparagus stem by longitudinal cuts one can recognize with the naked eye a vascular bundle parasitized thus by red streaks in the whitish tissue. It is possible to show thus that the fungus has grown from the point of infection as much as fifty centimeters downwards into the rootstock and 130 cm. upwards into the asparagus plant."

Observations in the Niagara Peninsula seemed to confirm the suspicion that the tremendous number of dying stalks, berries, and crowns in a mature plantation supports an extremely diverse fungus flora in which the genus *Fusarium*, with its marked saprophytic propensities, may be well represented. Therefore, the frequent observation of the presence of fusaria in isolates from lesions at stem bases and on the surface of stalks is at most coincidental and may give little indication of the true identity of the pathogen of seedling blight.

Symptoms of the Disease

In the field, the most prominent symptoms are on the older seedlings, the affected ones being stunted, yellowed, and wilted (Figs. 8-11). Blank spaces in the rows indicate pre-emergence blight. The wilt symptoms seem to be associated with a complete collapse of sections of the primary root (Fig. 13), while older seedlings with stunted and yellowed shoots usually have firm reddish-brown elliptical lesions of varying size on their roots. The seedling crowns may, through the activity of their laterally oriented apical meristems, produce fresh shoots of increased diameter and vigor. Small lesions on older crown roots are associated frequently with the points of attachment of lateral roots (Fig. 12), whereas those on primary roots may be located at any point between the hypocotyl and the growing point.

In plantations which had been set with crowns from a diseased seedling bed, misses and stunted plants were frequent. Such plants may have eventually constituted a major factor in reduction of yield of plantations sufficiently mature for cutting.

Under greenhouse conditions, symptoms of wilting of seedlings were much more common, and they could be used as an index of the degree of infestation of given samples of soil after seedlings had been grown in them. These symptoms prevailed during the interval between germination of the seed and the extrusion of lateral roots.

Pathological Histology

During the stage in which the primary root grew and developed laterals, a water-soaked grayish type of lesion (Fig. 13) was predominant. However, a microscopic examination of unstained roots mounted in water revealed the presence of small brownish elliptical areas conforming in shape to the orientation of the elongating cells of the young root.

The secondary roots had an outer layer of thick-walled cells which was lacking on the primary root and its laterals. Lesions on these secondary roots were easily visible to the unaided eye, and were usually elliptical in shape. They seemed to represent a primary stage in the development of the disease at the points of origin of the lateral roots. On still older seedlings, the lesions had lost their elliptical shape and firm consistency and became elongated, sunken, and brownish (Fig. 10).

Sections cut from all three types of lesions, water-soaked, firm-elliptical, and sunken-discolored, were examined in both the stained and the unstained condition. Examination of roots, either stained in lactophenol and cotton-blue, or unstained and crushed to permit a better view of the tissue underlying the epidermis, showed masses of fungus hyphae in the cortical tissue. Microconidia resembling those of *Fusarium* were seen floating freely in the mounts. Fig. 14 shows a macroscopic view of an infected primary root dissected to demonstrate the disintegrated cortex and intact stele.

In lesions produced on secondary roots, hyphae were located in both intercellular and intracellular spaces (Figs. 18 and 19). The reddish-brown color about them was found to belong to a layer of cells resembling a periderm, formed underneath the epidermis. This layer was semicircular in cross section and it characteristically delimited the area of fungal invasion (Fig. 15). Hyphae could be located in the peripheral areas between the cells and the hyphae extended for distances of several hundred microns in sections made lengthwise. Those in the central part occupied a cavity formed presumably by the shrinkage of cells which had been attacked and killed. Occasionally, lateral hyphae were found entering still intact cortical cells by means of a constriction and padlike swellings on both surfaces (Fig. 21).

The course of infection was traced by examining several hundred rootlets. Penetration is appressorial and direct and takes place between the walls of the epidermal cells, but rarely directly through the outer walls. The site of these penetrations is in the meristematic region of the root tip. Hyphae may also penetrate the stomata of the hypocotyl and of the coleoptile. As a result of artificial inoculation, microlesions were produced on the scales or reduced leaves on shoots above the surface of the soil and these were traced to stomata as their points or origin. The cell, or cells, at the point of penetration in the meristematic region, do not elongate further, though the adjoining cells continue to grow. Therefore, a puckered appearance of the epidermis of laterals under the microscope is an indication of infection. Such stunted cells take a deeper stain with cotton-blue, presumably because of the greater permeability of their walls. After the hyphae penetrate the root tip, they

may become intracellular almost immediately. In the fully differentiated part of the root, they extend, usually intercellularly, in lateral and vertical directions. The fungus seems to be remarkably variable in its manner of growth on the surface of the root and within it, and forms numerous swellings as it takes a tortuous course through the cortex. These swellings may be interpreted as appressoria (Fig. 22) in that their formation apparently precedes the passage of an extremely small intercellular space, or the penetration of a cell wall. The colonization of the host by the pathogen is limited to the cortical tissue.

An infection experiment was performed in which the lesions visible with the aid of a dissecting microscope were considered as indications of the presence of infection courts. Surface-sterilized seed of a susceptible variety of asparagus was planted in pots of sterilized soil and allowed to germinate to the stage at which lateral roots protruded. One hundred seedlings were inoculated with a spore suspension of the pathogen and incubated on moist filter paper in Petri dishes. After one week, lesions were visible on the root tips of 66 seedlings, and on the hypocotyls of four, while 34 remained apparently healthy. These results indicate that most of the infections which occurred were initiated in the region of the root tip, which is unprotected by either an enduring coleoptile or by thick walls of cellulose. They also suggest, however, that the pathogen may take advantage of natural openings on more mature parts of the seedling.

In mature crowns, fungus hyphae have been observed in and even isolated from the stelar elements; but none of the isolates from these tissues were capable of infecting seedlings or of penetrating directly in the manner described. The discolored vascular tissue was observed most frequently in direct connection with a wound, such as a cut which had severed the root completely. Therefore, it is suspected that such fungi were wound parasites.

Incidence of the Disease in Ontario

A survey, undertaken in the summer of 1948, was conducted concurrently with numerous attempts to isolate the pathogen. Samples of surface soil were collected from various mature plantations in the Niagara Peninsula in the vicinity of St. Catharines and Vineland. During the course of the survey, the approximate age of the plantations visited was noted. Samples were taken also from the roots of "wild" or "volunteer" asparagus, which was found growing along many fences and roadsides. Some were taken from the sods of clover, couch grass, and oats in order to investigate the possibility of the pathogen being indigenous to the Peninsular soils.

Each sample consisted of about 12 quarts of soil and was composed of at least 12 subsamples taken from various points in the plantation. Those taken from volunteer asparagus crowns were necessarily smaller. After composting and partial drying, each sample was put into a flat of dimensions approximately 18 in. × 12 in. × 3 in. Two hundred surface-sterilized seeds of a known susceptible variety, V-35 (Viking), recently introduced by the Horticultural Experiment Station, Vineland, were planted in each soil sample. The

following standard procedure was developed. Each lot of 200 seeds was soaked in distilled water for 24 hr., rinsed, immersed in 1 : 1000 mercuric chloride solution for five minutes, rinsed twice in sterile water, and dried on filter paper; the seeds were then planted in 10 rows of 20 seeds each at a depth of half an inch. The pertinent data are summarized in Table I.

TABLE I

DISEASE INCIDENCE IN SELECTED SOIL SAMPLES IN 1948

Soil sample No.	Crop from which soil sample taken	Age	From 20 seeds planted, number of seedlings:			
			Emerged	Healthy	Damped off	Infected
1	Asparagus	2 years	101	16	21	64
2	Asparagus	2 years	111	1	6	104
3	Asparagus	5 years	111	0	60	51
4	Asparagus	10 years	124	0	10	114
5	Asparagus volunteer	—	131	0	47	84
6	Couch grass	—	142	140	0	2
7	Clover	2 years	140	140	0	0
8	Oats	3 months	124	116	2	6
6	Check (sterilized soil)	—	122	122	0	0

As revealed in Table I, the presence of asparagus on land for from 2 to 10 years previous to the planting of the seedling crop seems sufficient to make conditions favorable for seedling blight. The incidence of the disease is apparently at a very low level or is absent from soils occupied by couch grass, clover, or oats. Of striking interest is the degree of infestation apparently present in Sample No. 5 from volunteer asparagus crowns. Unless the pathogen is seed-borne, it must be concluded that the pathogen had been in the soil before the introduction of the seed, and had been favored by the presence of roots of the asparagus plants.

A survey of similar extent was made in seedling plantations in the summer of 1949 (Table II). At the time of collection, data concerning the crop history were assembled. With two exceptions, *E* and *I*, all plantations showing infestation were adjacent to older plantations or were located on land which had been cropped to asparagus at some time in the past. Samples taken from one bed (*E*) indicated that the pathogen was present in alfalfa sod before the planting of asparagus seed. This observation seemed to indicate that the alfalfa was serving either as another host or after being plowed down, as a preferred substrate. It may have been altering the microbiological balance in a manner favoring the survival and persistence of the pathogen.

The manner of persistence of the organism in the soil in the absence of its probable preferred host is particularly interesting. In two cases, *D* and *H*, asparagus had been plowed down about ten years before 1949 and the land had been cropped to sugar beets and raspberries, and to potatoes and tomatoes respectively. In both instances the pathogen was present. It should be

TABLE II

DISEASE INCIDENCE IN SELECTED SOIL SAMPLES FROM SEEDLING PLANTATIONS IN 1949

Location	Previous crop history					Number of seedlings from 200 seeds			
	1944	1945	1946	1947	1948	Healthy	Damped off	Infected	Emerged
A. Niagara-on-the-Lake (1)	Unknown	Unknown	Unknown	Strawberries	Potatoes	123	1	38	162
Niagara-on-the-Lake (2)*	Unknown	Unknown	Unknown	Strawberries	Potatoes	125	0	49	174
B. St. Catharines*	Potatoes	Strawberries	Strawberries	Strawberries	Potatoes	152	0	8	160
C. Pt. Dalhousie*	Strawberries	Strawberries	Strawberries	Spinach	Asparagus	98	0	61	159
D. Jordan*	Sugar beets	Raspberries	Raspberries	Raspberries	Raspberries	23	14	119	156
E. St. Catharines (1)	Unknown	Oats	Timothy	Oats	Lucerne	86	6	61	153
St. Catharines (2)*	Unknown	Oats	Timothy	Oats	Lucerne	32	29	81	142
St. Catharines (3)*	Unknown	Oats	Timothy	Oats	Lucerne	85	7	55	147
F. St. Catharines*	Peaches	Peaches	Peaches	Strawberries	Asparagus	125	2	35	162
G. St. Catharines	Strawberries	Strawberries	Strawberries	Tomatoes	Tomatoes	154	0	17	171
H. St. Catharines (1)*	Tomatoes	Potatoes	Tomatoes	Potatoes	Tomatoes	56	19	113	188
St. Catharines (2)*	Tomatoes	Potatoes	Tomatoes	Potatoes	Tomatoes	51	7	124	182
I. Vineland*	Carrots	Carrots	Carrots	Carrots	Carrots	168	0	2	170

* *A. (2) Asparagus plantation adjacent to seedlings.*
* *B. Asparagus plantation adjacent to seedlings.*
* *C. Two-year-old asparagus plantation adjacent to seedlings.*
* *D. Asparagus grown for 12 years previous to sugar beets.*
* *E. (2) Sample taken in fallow land adjacent to E. (1).*
* *E. (3) Sample taken in potatoes adjacent to E. (1).*
* *F. One-year-old plants in seed-bed adjacent to a mature plantation.*
* *H. (1) Cropped to asparagus previous to 1940.*
* *H. (2) Sample taken from potatoes adjacent to H. (1).*
* *I. An intercrop between plum trees.*

observed that a mature plantation contains a very large quantity of fleshy roots which decay only after long periods in the soil. Given such a substrate the pathogen could probably survive indefinitely. Later experiments showed that it grew readily on organic matter other than that provided by dead asparagus crowns. Attention must also be called to the fact that samples collected from apparently healthy seedling plantations also yielded a few diseased seedlings and thus indicated the presence of a few centers of infestation. Unless the pathogen is seed-borne, there is thus a suggestion that it is indigenous to Ontario soils.

Both surveys indicated a wide distribution of the pathogen in the asparagus-growing region of the Niagara Peninsula. Its concentration in certain locations appears to be governed by such factors as the age of the plantation, proximity to mature plantations, and the presence of preferred substrates other than asparagus.

Etiology

Most of the isolates made from lesions on mature crown roots and from diseased seedlings proved to be members of the genus *Fusarium*. Two types seemed to predominate: the one ochraceous or cream-colored and the other, white or pink. The latter colored the substrate a deep vinaceous red. Because of its consistent appearance in the marginal areas of lesions and its isolation directly from spores produced on diseased roots, the cream-colored organism was placed under suspicion. Subsequent inoculations and re-isolations proved it to be the pathogen of seedling blight.

It was at first impossible to reproduce typical field symptoms of the disease since the damping-off symptom appeared most frequently on seedlings one half to three inches in height. Therefore, a series of experiments were performed to compare the effects of varied concentrations of inoculum upon symptom expression. First, a corn meal and sand medium, prepared according to the formula of Riker and Riker (16), was added to soil at rates ranging from 1 to 5% by weight. Concentrations as low as 1%, even in unsterilized soil, supported an expression of pre-emergence blight different from that of field outbreaks. A second experiment was therefore planned in which the inoculum, which had been growing at 25° C. for two weeks, was stratified in layers half an inch deep at varying distances from the bottoms of the flats. The seed was planted at a depth of half an inch from the surface. After one month, a count of emerged and damped off seedlings was taken, and the data are presented in Table III.

The greatest amount of pre-emergence and postemergence blight occurred in the flat in which the inoculum had been introduced two inches from the bottom of the flat directly at the level of the seed. This suggests also that the percentage of seedlings emerged depends upon the degree of host development before contact was made with the inoculum. The high incidence of blight at the two inch or seedling level is correlated with the intimate association of the host and pathogen during germination and emergence.

TABLE III

THE EFFECT OF DEPTH OF STRATIFICATION OF INOCULUM IN STERILIZED SOIL UPON EMERGENCE OF ASPARAGUS SEED AND ON WILTING OF SEEDLINGS

Distance of the inoculum from bottom of the flats, in.	No. of seedlings	
	Emerged	Wilted
2½	192	33
2	165	66
1½	191	20
1	197	13
½	194	4

In an experiment employing spore suspensions of known concentration it was found that one yielding 15 spores per high power field (430× *ca.*) gave an ultimate symptom expression approximating that shown by seedlings grown under greenhouse conditions in naturally infested soil.

The Associative Effect of Another Fusarium

The ubiquity in asparagus fields of a *Fusarium* which produced a reddish pigment in culture was demonstrated by its repeated isolation from seeds and from dead asparagus stalks. This organism was identified as *Fusarium moniliforme* Sheldon. In 1949, a number of single-spore isolations were made from conidia which developed, following rain, in tufts or dense mats upon the lower portion of dead or dying stalks. Although the fungus had been presumed to be saprophytic under these conditions, it was demonstrated to be pathogenic to seedlings. It caused a distinct necrosis of the root tips (Fig. 9), but no cortical decay. The results of mixing inocula of two fusaria possessing an apparent specialization to substrates differing in structure are presented in Table IV.

TABLE IV

THE EFFECT OF MIXING INOCULA OF FUSARIA PATHOGENIC TO ASPARAGUS UPON EMERGENCE AND DAMPING-OFF OF ASPARAGUS SEEDLINGS

Fusarial strain	Concentration by volume	From 200 seeds planted, number of seedlings:			
		Emerged	Healthy	Damped off	Infected
Cortical pathogen	2%	33	0	22	11
F. moniliforme Sheld.	2%	114	4	6	104
Both: cortical pathogen + *F. moniliforme* Sheld.	1% + 1%	120	0	6	114
Sterilized soil	—	122	122	0	0
Naturally infested soil	—	114	83	1	30

Seedlings dug from the flat containing soil with mixed inocula exhibited a striking combination of symptoms. Most of the laterals on the secondary roots were killed or were replaced by streak lesions at their points of emergence. The significantly lower degree of pre-emergence blight where both inocula were used may have been due to the reduction from 2% to 1% in concentration by volume of inoculum of the organism primarily responsible for it. But the degree of reduction is out of proportion and suggests the possibility of antagonism between the two fusaria at their common points of entry, the root tips.

While *Fusarium moniliforme* Sheld. certainly did not cause symptoms of blight as it occurred on seedlings in the field, its presence as a ubiquitous saprophyte in asparagus fields, and on seed, cannot be ignored in view of its ability to stunt the growth of both root-apical and lateral meristems.

Description of the Causal Organism of Asparagus Seedling Blight

The type of spore produced on the host under natural conditions of infection has not been studied extensively. However, the following phenomena were observed consistently: (1) on streak lesions on six-week-old asparagus crowns held overnight in a refrigerator, macroconidia and microconidia were produced in abundance and could be detected easily in cross sections cut from the diseased roots; (2) microconidia were produced most abundantly upon the surface of shoots emerging from sterilized artificially infested soil; (3) chlamydospores were detected throughout the tissues (Figs. 16 and 17) of diseased seedlings which had become dried.

The causal organism was found capable of producing all its spore forms in culture on 2% potato-dextrose agar made according to the formula of Riker and Riker (16). However, an interval of two weeks was necessary before macroconidia could be produced in sufficient quantity to make identification possible. The following cycle of spore production was observed on soil agar. After two days of much-reduced vegetative growth, no conidia were apparent. After four days, a few one- or two-celled microconidia were apparent; after six days, macroconidia (Fig. 27) were present at the point of inoculation of the dish. The spore form most frequently produced at the edge of the colony was the microconidium, while those found between the edge and the center possessed one or two septa, or rarely, three. After two weeks, chlamydospores in clumps or chains of two and three appeared in both terminal and intercalary positions. Their numbers increased as the agar dried in the Petri dishes.

The cortical pathogen was identified by Dr. W. L. Gordon, of the Laboratory of Plant Pathology, Winnipeg, Man., as *Fusarium oxysporum* Schlecht. sensu Snyder and Hansen, and at the time he indicated its similarity to *Fusarium redolens* Wr. In a recent paper (11) Gordon proposed a new combination, *F. oxysporum* Schlecht. emend. Snyder & Hansen var. *redolens* (Wr.) to differentiate the species which Wollenweber and Reinking (23) designate *F. redolens* Wr. He felt that this distinction was justified because of certain characteristics such as spore shape and color of the substratum.

A culture of Dr. Gordon's *F. oxysporum* var. *redolens* was obtained and the growth of single-spore isolates of this organism and of the cortical pathogen were compared on potato-dextrose agar and on soil extract agar. During the first 10 days of culture, their color, odor, and gross appearance were virtually identical. The spores produced on P.D.A. were shorter and probably broader than those which are described in Gordon's recent paper (11). But as he maintained his isolates on potato-sucrose agar certain differences might be expected. These were not evident when P.D.A. or soil extract agar was the common medium Therefore the cortical pathogen of asparagus is assigned to *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen var. *redolens* (Wr.) Gordon.

It must be noted, however, that Gordon's culture was incapable of causing any disease after the inoculation of seedling asparagus. It is therefore concluded that parasitic and saprophytic strains of this species exist.

Fig. 26 shows single-spore cultures of the various fusaria found or reported to be pathogenic to asparagus. It is evident that *F. oxysporum* var. *redolens* is distinct at least in its gross morphology from the other representatives of the genus illustrated.

The Effect of Different Carbohydrates on the Growth and Sporulation of F. oxysporum var. redolens and of F. moniliforme Sheldon

In view of the fact that saprophytism may be an important factor in the determination of inoculum potential, the response of the pathogen to substrates of carbohydrate nature was investigated. The two fungi found to be pathogenic to asparagus in the Niagara Peninsula, *F. oxysporum* var. *redolens* and *F. moniliforme*, were grown in liquid culture in solutions containing varying quantities of glucose, sucrose, and cellulose, and on starch agar.

(*1*) *Glucose*

A standard formulation of mineral nutrient solution was used throughout the experiments involving the use of liquid culture. This solution had been used by Lochhead *et al.** (14) in their studies on the nutrition of various members of the microflora associated with certain soil-borne diseases. Glucose was added in the quantities of 0.1 gm., 0.4 gm., 0.7 gm., and 1 gm. to lots of the basal formulation. One lot was left without glucose as a check. These were dispensed in 2½-cc. amounts into bacteriological microtubes in quintuplicate. The tubes were sterilized by heating in the autoclave at a pressure of 15 lb. for 20 min. The pH was checked after sterilization and found to be close to 6.8. The tubes were then inoculated with a spore suspension of each of the organisms at the rate of one 2-mm. loopful per tube. The spores of *F. oxysporum* var. *redolens* were considerably larger than those of *F. moniliforme*, which produced no macroconidia. Consequently, it is possible that there is actually less growth potential present in the inoculum of the latter organism. Tubes of sterilized distilled water were also inoculated with each

* *These are the materials and the quantities in which they were used:* K_2HPO_4, *1 gm.;* KNO_3, *0.5 gm.;* $MgSO_4$, *0.2 gm.;* $CaCl_2$, *0.1 gm.;* $NaCl$, *0.1 gm.;* $FeCl_3$, *0.01 gm.; water, 1 liter.*

of the fungi, in order to determine whether anything was present in the water alone which would be capable of supporting growth. The tubes were kept at 25° to 26° C. for a period of 10 days before observations were taken.

After three days, clumps of mycelium were noted on the bottom and on the sides of most of the tubes. This mass finally formed a weft of hyphae on the surface of the solution. After 10 days, growth in the various concentrations of glucose was recorded by an arbitrary scale ranging from 0 to 4, with 0 representing no growth and 4 indicative of the greatest amount of growth obtained. The records for growth of the two organisms in glucose are presented with those for growth in sucrose in Table V.

Observations were made also on the degree of spore production in this series. In *F. moniliforme*, microconidia appeared at a glucose concentration of 0.1 gm. per liter but did not become profuse before the level of 0.7 gm. per liter was reached. For *F. oxysporum* var. *redolens*, perceptible sporulation began at the 0.4-gm. level, but was profuse only in the 1-gm. concentration. In the lower concentrations of 0.1 gm. and 0 gm. per liter, growth of hyphae was chlamydosporous. It was interesting to note that *F. moniliforme* seemed incapable of producing chlamydospores at even the lowest concentrations.

(2) Sucrose

The technique of preparation of the solutions and the cultures of the organisms was the same as that for the glucose series. However, the weight of sucrose per liter was one half that of the glucose at each concentration, as it would be necessary to add the equivalent number of moles per liter in order to compare responses to the two carbohydrates correctly. The results are also summarized in Table V.

The degree of sporulation of *F. moniliforme* was considerably less than in the glucose series. Macroconidia as well as microconidia were present in the

TABLE V

THE EFFECT OF GLUCOSE AND SUCROSE ON THE GROWTH OF *F. moniliforme* AND *F. oxysporum* VAR. *redolens*

Organism	Glucose (gm. per l.)	Growth (av. of 5 tubes)	Sucrose (gm. per l.)	Growth (av. of 5 tubes)
F. moniliforme	1	4*	0.5	3
	0.7	3	0.35	2
	0.4	2	0.2	1
	0.1	Tr*	0.05	Tr
	0	Tr	0	Tr
F. oxysporum var. *redolens*	1	4	0.5	4
	0.7	3	0.35	3
	0.4	2	0.2	2
	0.1	Tr	0.05	1
	0	0	0	Tr

** Nos. 0 to 4 represent an arbitrary scale: 0 represents no growth and 4 the maximum growth obtained; Tr, a trace of growth.*

liquid cultures, in spite of their apparent absence from the spore suspension which constituted the inoculum. Macroconidia were also absent in the soil agar or in the P.D.A. cultures of the particular isolate used in these tests. In the case of *F. oxysporum* var. *redolens*, the trend in the degree of sporulation in the various concentrations was similar to that in the glucose series. Chlamydospores were also present in concentrations of 0.05 and 0.2 gm. sucrose per liter, but were relatively infrequent at 0.35 gm.

(*3*) *Cellulose*

Pieces of No. 5 Whatman filter paper weighing 0.5, 0.35, 0.2, and 0.05 gm. were added to 200 cc. flasks containing 75 cc. of the standard nutrient solution. After preliminary sterilization, the fungi were inserted in the flasks in the usual manner and incubated for 10 days at 25° to 26° C. After two days, a slight fuzz was noted on the surface and on the cut edges of the paper in the flasks inoculated with *F. moniliforme*. By the end of the 10-day period, there was a dense bed of spores on the exposed surfaces of all paper in the concentrations of 0.5, 0.35, and 0.2 gm. per liter whereas the fuzz produced by *F. oxysporum* var. *redolens* was relatively sparse. When the filter paper was being removed from the flasks, it was noted that the paper colonized by *F. moniliforme* possessed a much greater tendency to shred. It may therefore be concluded that as its growth was greater the latter organism utilized cellulose more efficiently.

(*4*) *Starch*

The use of liquid media containing soluble starch would not have permitted the taking of comparable readings because of excessive milkiness. Therefore, starch agar was prepared. The salts used in the standard formulation were mixed with 1 gm. of potato starch by heating in the autoclave for 20 min. at a pressure of 15 lb. Then the agar was melted and decanted into the salt solution and the mixture was again autoclaved for 20 min. at a pressure of 15 lb. The reaction of the medium after sterilization was found to be pH 6.6. This was adjusted with KOH to pH 7.0. The plates were poured and inoculated in quadruplicate and incubated at room temperature (approx. 25°–26° C.) for four days. They were then transferred to a refrigerator for one week at 5° C., to stop the growth of the mycelium. By this treatment, the zone of starch utilization which appeared after the plate had been flooded with a 2% solution of IKI was considerably greater than that of plates left at room temperature. The average diameter of the colonies and the width of the starch-free zones were 31.25 mm. and 6.0 mm. respectively for *F. moniliforme*, and 29.75 mm. and 7.5 mm. for *F. oxysporum* var. *redolens*.

It is apparent that under the environmental conditions prevailing during the experiments, the two fungi are capable of using glucose equally well, that *F. oxysporum* var. *redolens* uses sucrose more readily than *F. moniliforme*, that *F. moniliforme* breaks down cellulose more rapidly than *F. oxysporum* var. *redolens*, and that *F. oxysporum* var. *redolens* can hydrolyze starch more easily than *F. moniliforme*, although the latter organism makes greater linear growth on the starchy medium.

The formation of chlamydospores by *F. oxysporum* var. *redolens* in the lowest concentrations of glucose and sucrose suggests that this organism is capable of responding to conditions unfavorable for growth. In this case, lack of sufficient carbohydrates seems to be the factor involved. It is suggested that similar conditions of starvation may bring about the formation of chlamydospores in naturally invaded roots. The factors involved there may be the competitive action of other organisms entering the lesions, or lack of moisture. Indeed, anything which might prevent the organism from using the supply of carbohydrate might lead to a cessation of growth and to the formation of chlamydospores. It is interesting to note, however, that *F. moniliforme* seemed incapable of forming chlamydospores at even the lowest levels of sugar, but must have possessed some other means of stopping growth in preparation for a "rest period".

The Effect of Temperature on the Growth and Sporulation of F. oxysporum var. redolens

Growth, in the Fungi Imperfecti, is a combination of linear increment of the hyphae and the production of spores on the mycelium thus formed. Therefore the use of a translucent medium, soil agar, was included in these investigations. Such a medium permitted the observation of the degree of sporulation which took place in response to varying degrees of temperature.

Twenty-eight plates each of soil agar and potato-dextrose agar were inoculated in the center with a disk of inoculum from the advancing edge of a single-spore colony of the pathogen growing on P.D.A. Four plates of each medium were disposed at each of the temperatures 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. The media were at a pH of about 6.4. After one week the plates were examined and the colony diameters were measured. No great discrepancy existed between the optimal temperatures on the two media. The curve between 25° and 30° C. was ascending on P.D.A. and descending on soil agar.

The effect of temperature upon the degree and type of sporulation on soil agar is illustrated in Fig. 32. The manner of growth on P.D.A. did not permit observations on the relative degree of sporulation. It was noted, however, that at 5° C. no growth took place for the duration of the experiment, while at 35° C. a pulvinate type of colony (Fig. 31) revealed a vesiculoid sporeless type of growth similar to that produced on soil agar. Between these two extremes, microconidia and macroconidia in undetermined proportions were present. Therefore it was concluded that the maximum temperature for normal growth lay somewhere between 30° and 35° C.

Optima for microconidium and macroconidium production seemed to be 15° and 25° C. respectively. Therefore, it was decided that the effect of shifting soil agar plates containing growing cultures of the pathogen from one temperature to the other should be investigated. After three days' development, two plates were shifted from 25° C. to 15° C. and vice versa. They were examined after one week. The 15° C. plates which had been shifted to 25° C. showed a marked increase in the production of macroconidia at the

line where the advancing margin of the hyphae were located at the time shifting took place (Figs. 33 and 34). The 25° C. cultures shifted to 15° C. showed a general decrease in the quantity of sporulation since most of the plates had been covered. The effect of the shift on spore type was not marked, but the relative number of macroconidia seemed to be reduced.

It was observed that microconidia were produced in plates held for three weeks at 10° C., while macroconidia were present, in relative abundance, in cultures left for the same length of time at 15° C. They were produced at the point of introduction of the inoculum at the center of the plate. Therefore, the apparent differences in optima for the two types of spores are considered to be due to the different rates at which the colonies approach sporulation age at the various temperatures.

The Effect of Temperature and Moisture upon Growth Rate in Soil Tubes

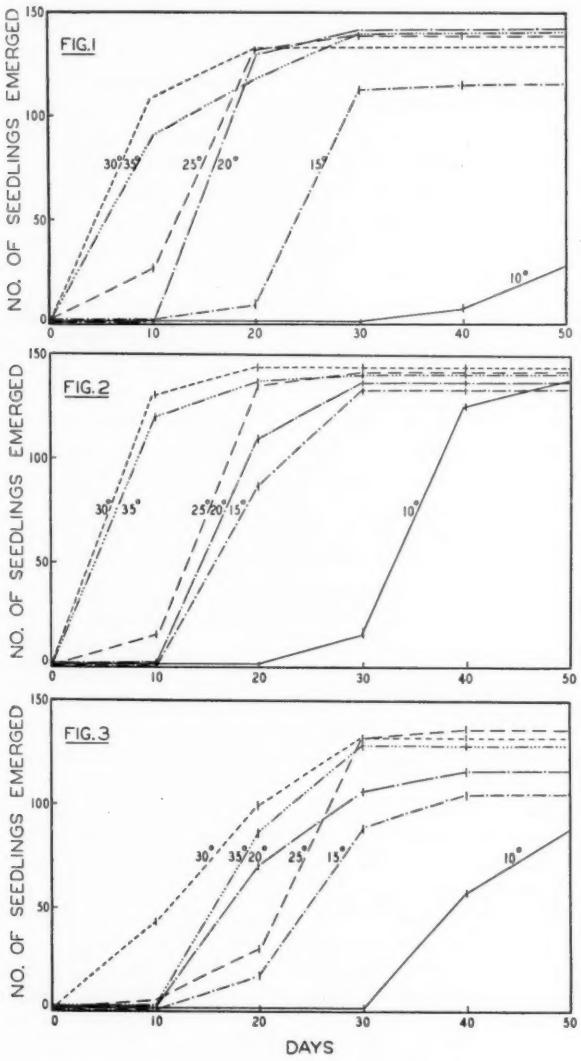
Tubes four inches long, open at both ends and with an inside diameter of about 2 mm. were filled in quadruplicate with soil at three different moisture levels. This soil had been dried in an oven and before being placed in the tubes was adjusted by the addition of sterile water to 20%, 50%, and 80% of its moisture-holding capacity. At the 80% level it was wet to the extent of being puddled, while that at 20% and 50% was quite friable. The soil in all tubes was tamped down with a glass rod. The tubes were autoclaved for one hour at a pressure of 20 lb. and were inoculated at one end with a disk of inoculum cut from a culture of the pathogen growing on soil agar. The ends were plugged with cotton batting previous to sterilization. The tubes were placed in clay saucers at the bottom of the canisters in the Wisconsin soil temperature tanks, four at each of the temperatures of 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. Humidity inside the cans was maintained by keeping about half an inch of water on the bottom, and was assumed to remain close to 100%. After one week, the linear development of the mycelium on the inside of the tubes was visible with the aid of a dissecting microscope, and measurements were taken.

No significant inhibition or stimulation of development was noted at any of the moisture contents used. A slight difference, however, appeared between the amount of linear growth in the soil held at the high and at the low moistures, particularly over the range from 25° to 30° C. Although the tubes containing the soil at the low soil moisture may unavoidably have gained some moisture under the conditions of the experiment, the magnitude of the difference between them at the end of the period was still that of wet soil compared with dry soil.

Epidemiology

The Effect of Temperature and Moisture upon the Development of Asparagus Seedlings in Sterilized Soil

Figs. 1, 2, and 3 show the effect of 10%, 35%, and 60% soil moisture on the emergence of seedlings from sterilized soil at various temperatures. It is evident from these curves that the most rapid emergence, at all temperatures,



FIGS. 1-3. The effect of temperature on the rate of emergence of asparagus seedlings in sterilized soil held at low moisture (10%) (Fig. 1), medium moisture (35%) (Fig. 2), and high moisture (60%) (Fig. 3).

took place in the soil held at the medium moisture level and that the optimum temperature for emergence is 30° C. Emergence is slightly slower at 35° C., much slower and at about the same rate at 15°, 20°, and 25° C. whereas the greatest retardation is between 10° and 15° C.

The general effect of low and high soil moistures is to retard the rate of emergence. The effect is indicated by a shifting of the ascending lines of the curves to the right and by a depression of the angle of ascent. There are, however, two exceptions. At 20° and 25° C., no retardation of emergence is evident at the low level of moisture, and at 10° C., low soil moisture depressed germination much more than the high soil moisture.

The Effect of Temperature and Moisture on Disease Incidence in Seedlings Germinating in Naturally and Artificially Infested Soils

Soil for these experiments, obtained from the original bed of the Viking (V-35) variety at Vineland was passed through a coarse sieve. Soil for artificial infestation was prepared by sterilizing naturally infested soil for one hour at 15–20 lb. in the autoclave. A spore suspension was made by washing two-week-old slant cultures of the pathogen on P.D.A. with 50 cc. of sterile water and the soil was infested at the rate of 50 cc. of spore suspension per canister. A 6 in. pot was inverted in the bottom of each canister to supply drainage. The canisters were filled to within 8 in. of the top with sterilized sand, and 6 in. of soil was added. Because of limited space, two consecutive experiments were conducted. In the first, two cans each of sterilized artificially infested soil and of naturally infested Vineland soil were held at 35% and at 60% soil moisture; in the second, two canisters each of artificially infested soil and of naturally infested soil were held at 10% and at 35% moisture. Seventy-five seeds of the variety Viking (V-35) were sown in each of the canisters at a depth of half an inch.

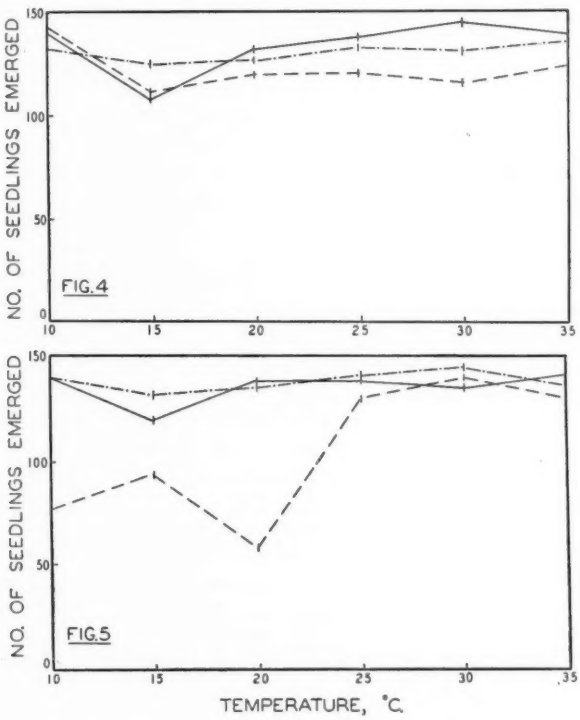
The water temperature in the Wisconsin tanks was maintained at 10°–12°, 15°–17°, 20°–22°, 25°–27°, 29°–31°, and 33°–35° C. Although the differential settings on the thermostats permitted a range of variation narrower than two degrees, a check of the temperature of the soil at the center of the canister, at the half-inch depth at which the seeds were planted, revealed that it was rarely the same as at the outside of the canister. In the tanks being operated at temperatures more than 5° C. above or below greenhouse temperature (20° C.) the temperature at the center of the canister was greater or less than that at the outside of the canister by two degrees. During the period of germination and the initial stages of emergence an attempt was made to overcome this discrepancy by covering the canisters with clay saucers of the same diameter as the canisters. After emergence, the saucers were removed to admit light to the seedlings. It seems probable that by this time the primary roots had grown through the layer of soil in which the greatest variation in temperature took place, and that further attack on the plants was made under more constant conditions.

Atmospheric humidity and temperature were not controlled during this experiment, except by ventilating and shading the greenhouse by whitewashing

the glass. The canisters were sprinkled daily to keep the soil moisture close to the initial low, medium, and high levels of moisture. From time to time, small quantities of soil were removed from the canisters, weighed, dried in a hot-air oven, and weighed again. This simple check showed that the low, medium, and high levels of moisture corresponded closely with the 10, 35, and 60% levels striven for. After six weeks, the surviving seedlings were dug and examined. The number of seedlings which emerged and damped off are recorded graphically in Figs. 4, 5, 6, and 7.

In naturally infested soil a reduction in emergence is noted (Fig. 4) and 10° to 20° C., at the low and high soil moisture. A more marked reduction of emergence occurred at the same range of temperature in artificially infested soil (Fig. 5), while in both soils emergence at medium moisture was at about the same rate.

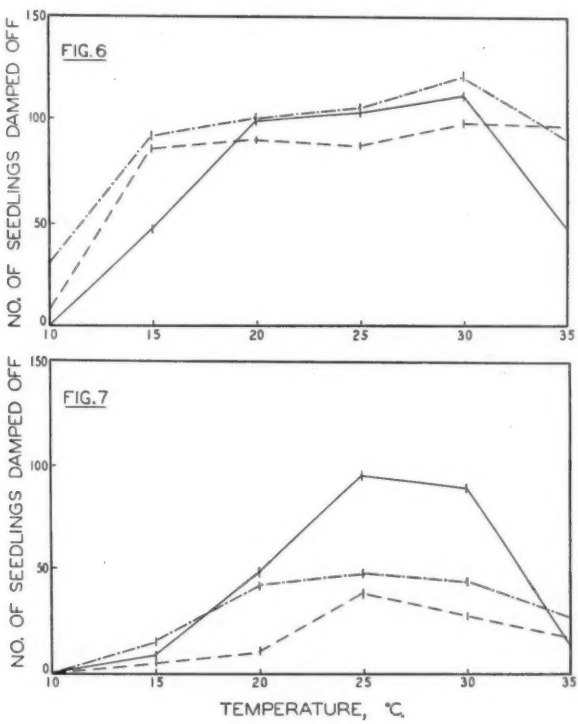
The optimum temperature for damping off in naturally infested soil was between 20° and 30° C., at low soil moisture. At the medium and high



FIGS. 4 and 5. The effect of temperature on the degree of emergence of seedlings in naturally infested soil (Fig. 4) and artificially infested soil (Fig. 5) held at three different levels of moisture. (————) low soil moisture, (-·-·-·) medium soil moisture, (– – –) high soil moisture.

moistures it was prevalent from 15° to 35° C. (Fig. 6). In artificially infested soil, low soil moistures yielded the highest number of damped off seedlings between 20° and 35° C. (Fig. 7).

Low soil moisture tends to retard the rate of emergence between 10° and 20° C. (Figs. 1 and 2). It has already been shown that soil moisture within the range of 20 to 80% of water-holding capacity had little effect on the growth rate of the pathogen. Therefore it is suggested that the low moisture predisposes the seedlings to pre-emergence blight by prolonging the phase of emergence. It is concluded from the reduction in total numbers emerged between 10° and 20° C. that this phenomenon took place in both naturally and artificially infested soils (Figs. 4 and 5). The curves indicating the numbers damped off at low soil moisture (Figs. 6 and 7) show less damping-off between 10° and 20° C. than at any of the other levels. The explanation offered is that fewer seedlings emerged and reached the stage at which damping-off usually occurred.



FIGS. 6 and 7. The effect of temperature on the degree of damping-off of seedlings in naturally infested soil (Fig. 6) and artificially infested soil (Fig. 7) held at low, medium, and high levels of moisture.

High soil moisture caused a more marked decrease of emergence of seedlings from sterilized soil at all temperatures investigated, except at 10° C. (Fig. 3). The effect on pre-emergence blight was again evident at the 10°–20° C. range, especially in artificially infested soil (Fig. 5). In artificially infested soil a much lower number of seedlings emerged and reached the damping-off stage. The percentage of damped off seedlings is lower in soil held at a high moisture level than at either medium or low levels. The increased water supply provided by a high soil moisture to damaged parts of the seedlings may have reduced damping-off to a certain extent.

The effect of moisture was not nearly so marked in naturally infested soil as in artificially infested soil. It was noted that considerably more water was required to keep the naturally infested soils up to their required moisture levels. One reason for this may be the alteration of the structure of the soil by sterilization and also of its water-absorbing capacity. The absence of antagonistic organisms in sterilized soil may account for the rapid invasion of the medium by the pathogen. The effect of varying moisture levels may then be more pronounced if disease incidence is taken as a criterion.

The Effect of Temperature on the Rate of Infection Following Contact of the Host and Pathogen

The initiation of infection following contact of the host and the pathogen was investigated by a special technique. Seedlings which had been grown in sterilized soil and which had not yet acquired lateral roots were dipped in a spore suspension of the pathogen and placed in Petri dishes lined with moist filter paper. Ten seedlings were placed in each dish and were exposed to temperatures of 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. After 24 hr. no lesions had occurred at 5°, 10°, 20°, or 35° C., while there was one at 15°, five at 25°, and four at 30° C.

These observations suggest that the effect of temperature upon the rate at which infection took place parallels closely the effect of temperature on the growth of the pathogen. It is unlikely, however, that infection takes place under natural conditions from spores germinating upon the surface of the roots. It is more probable that hyphae of the fungus make contact with the root after having grown from a spore or a mycelial fragment located at a point in the soil some distance from the roots.

The Possibility of Seed-borne Transmission of Seedling Blight

After the first occurrence of the disease in 1945, a number of growers suspected that the causal organism was seed-borne. The general opinion was that it had been transmitted by a lot of seed distributed by the Vineland Horticultural Station at the time of introduction of the V-35 selection of Mary Washington. Therefore it was considered necessary to investigate the possibility of its presence on the seed.

(*1*) *The Attempted Isolation of the Pathogen from Seed*

Eighty shrunken seeds were culled from each of the varieties V-35 and Mary Washington. They were soaked in sterile water for 24 hr., surface-

sterilized for three minutes in mercuric chloride (1 : 1000), given two rinsings in sterile water, and placed with sterile forceps on 2% P.D.A. which had been acidulated by the addition of three drops of 10% lactic acid to each plate. A count of the organisms present was taken after one week. Colonies of species of *Alternaria* and *Penicillium* were found but none of the pathogen appeared. It is also interesting to note that during the course of an attempt to grow asparagus under aseptic conditions on P.D.A. in Petri dishes and on nutrient agar, some 500 healthy seeds were plated out after customary surface sterilization. None of these produced any colony resembling the pathogen.

(2) Attempted Isolation of the Pathogen in Washings from the Seed Coats

Two hundred seeds each of the varieties V-35 and Mary Washington were shaken thoroughly in 100 cc. of sterile water and left standing for 12 hr. in order to initiate growth of any fungi present. The supernatant suspension of debris was then poured off and dilutions of 1 : 100, 1 : 500, and 1 : 1000 were made. One cubic centimeter of each of these dilute suspensions was poured into plates containing melted acidulated 2% P.D.A. The plates were incubated at 25°–27° C. for six days. Colonies of fusaria were subcultured and examined but none developed characteristics typical of the pathogen, *F. oxysporum* var. *redolens*.

The Rate of Invasion of Non-infested Soil by the Pathogen

The rate of invasion of an orchard soil which had produced originally only two infected asparagus seedlings (Table VI) was studied. Successive lots of surface-sterilized seed of the Viking (V-35) variety were planted in a flat of this soil in the greenhouse at intervals of six weeks. No attempt was made during this period to modify the natural fluctuations of this environment. The lower temperatures throughout the winter months lengthened the period necessary for emergence and may thus have predisposed the seedlings to pre-emergence blight. The records of disease incidence throughout seven successive "crops" of asparagus are presented in Table VI.

TABLE VI

THE EFFECT ON DISEASE INCIDENCE OF CROPPING AN ORCHARD SOIL TO SUCCESSIVE PLANTINGS OF ASPARAGUS

Planting	From 200 seeds planted, number of seedlings:			
	Emerged	Healthy	Damped off	Infected
1	170	168	0	2
2	161	150	0	11
3	195	159	0	36
4	189	33	67	89
5	190	11	84	95
6	186	0	101	85
7	197	0	103	94

The general trend in this cropping series was a rise in the number of infected individuals with each successive planting up to the sixth. During the seven plantings, the pathogen may have undergone a simple quantitative increase brought about by the addition of the substrate it seems to colonize most frequently. Also the microbiological balance in the soil may have been shifted to become more favorable for the pathogen by the action of the roots on the microflora in the surrounding soil. These results indicate clearly how a perennial crop such as asparagus accumulates, in the course of successive years of cropping, a formidable concentration of the pathogens peculiar to it. It would be dangerous, however, to predict displacements in the same direction of the microbiological balance in all types of soil and crop rotations.

The Effect of Various Plant Residues on Disease Incidence

Naturally-infested soil was collected from the original plantation of the V-35 variety at Vineland and, after thorough mixing, was put into flats. Three flats each of soybeans, oats, potatoes, red clover, alfalfa, and asparagus were planted. The quantity of seed of each crop sown per flat varied, but was sufficient to give adequate cover after a few weeks' growth. At this time the seedlings were cut just above the soil level, chopped finely, and mixed thoroughly with the soil in the flat. The soil in two of the three flats of soybeans, oats, etc., in which had grown this first crop was replanted immediately to a second crop. This procedure was repeated to give flats of soil possessing one, two, or three crops turned under. One week after each incorporation, 200 surface sterilized seeds of the Viking (V-35) variety were sown in each flat. The records of damped-off, infected, and healthy seedlings are presented in Table VII.

TABLE VII

THE EFFECT OF VARIOUS COVER CROPS AS COMPARED WITH ASPARAGUS ON DISEASE INCIDENCE IN NATURALLY INFESTED SOIL

Crop	Number of plantings	Emerged*	% Healthy	% Damped-off	% Infected
Asparagus (5 weeks)	1	191	0.0	57.6	42.4
	2	152	0.0	49.3	51.7
	3	161	0.0	57.7	42.3
Soybeans (3 weeks)	1	158	2.5	60.8	36.7
	2	157	0.0	53.5	46.5
	3	75	4.0	74.8	21.2
Oats (3 weeks)	1	193	2.1	68.8	29.1
	2	183	1.7	65.6	32.7
	3	103	1.9	61.2	36.9
Potatoes	1	185	0.0	70.9	29.1
	2	143	0.0	60.1	39.9
	3	176	0.0	80.1	19.9
Red clover (5 weeks)	1	186	0.0	59.1	40.9
	2	138	7.3	60.2	32.5
Alfalfa (5 weeks)	1	128	8.0	90.6	1.4

* *From 200 seeds planted.*

If the total number of seedlings which failed to emerge after each planting is taken as a criterion, the most rapid increase in disease incidence occurred in the soybean and oats series, compared with that of asparagus. However, the red clover series shows a remarkable reduction after two crops, and the alfalfa series after only one crop. In relation to the amount of material incorporated, the increase of disease in the potato series seems to be disproportionately low. Also, in view of the fact that asparagus is the preferred host, it seems illogical that the increase in disease incidence in this series should take place at a slower rate than with the other crops.

It is interesting to note that the rate of decrease of emergence in the three leguminous crop series, soybeans, red clover, and alfalfa, was relatively greater than in the nonleguminous series, oats, potatoes, and asparagus. While it is not clear what is responsible for this response, it does suggest that the pathogen is favored in some way by the leguminous residues. This observation may throw some light on the occurrence of the pathogen in land which had never been cropped previously to asparagus but which had borne a crop of alfalfa for a year previous to that in which the disease appeared in a seedling plantation (Table I).

Host Range

The host range of the pathogen was investigated by planting the various suspected hosts in naturally and artificially infested soils. Seed of onions (*Allium cepa* L.), spinach (*Spinacia oleracea* L.), lucerne (*Medicago sativa* L.), gladiolus (*Gladiolus* sp.), variety Picardy, *Asparagus plumosus* Baker, *Asparagus sprengeri* L., oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum vulgare* L.), rye (*Secale cereale* L.), and corn (*Zea mays* L.) were surface sterilized and planted. Of this group, only the roots of gladiolus and *Asparagus plumosus* Baker showed lesions which yielded the pathogen after attempts at re-isolation. This suggests a specialization to the Liliaceae of a soil-inhabiting fungus. The colonization was cortical and emphasizes further the possibility that this organism may be specialized to that particular tissue.

Control

(*1*) *Crop Rotation*

In view of the proved saprophytic capabilities of the pathogen, crop rotation cannot be regarded as a feasible means of control. It is evident that following a heavy natural infestation brought about by growing asparagus the organism can persist for years in the presence of diverse intervening crops. Furthermore, the incorporation of green manure or cover crops in a limited series of experiments leads in every instance to an intensification of disease incidence.

(*2*) *Selection of the Seedbed*

Surveys have shown that in proximity to mature asparagus, plantations are likely to become diseased if seed is planted on them. Furthermore, suspicion

TABLE VIII

THE EFFECT OF PLACEMENT OF ARASAN ON DISEASE INCIDENCE IN NATURALLY INFESTED SOIL

Planting	From 200 seeds planted, number of seedlings:			
	Emerged	Healthy	Damped off	Infected
Check (sterilized soil)	192	192	0	0
Check (naturally infested soil)	167	0	104	63
Seed coated	196	0	159	37
Bands on both sides of seed	184	0	146	38
¼ in. directly below the seed	185	0	86	89
¼ in. directly above the seed	193	0	136	57

is cast upon alfalfa residues. It is therefore advised that land with such a crop history, even though as much as ten years have intervened, be avoided in the establishment of seedling plantations.

(*3*) *Chemical Treatment of the Seedbed or the Seed*

The results presented in Table II indicated that the greatest amount of damping-off occurred in the flat in which the inoculum was introduced at the level of the seed. This evidence corroborated the finding that the principal loci of infection are the root tips. It seemed advisable, therefore, to investigate the matter of optimal placement of the chemicals in the seedbed. The material chosen was Arasan in the 50% wettable formulation, applied at the rate of 5 gm. per flat by the methods recorded in Table VIII. Surface-sterilized seed of Viking (V-35) was planted at the usual rate of 200 seeds per flat at a depth of half an inch in naturally infested Vineland soil. After six weeks, the seedlings were examined for the presence of disease.

It is evident that placing the Arasan a quarter of an inch directly below the seed was the most effective treatment against damping-off. In this case, the Arasan was introduced into the area in which the greatest degree of infection was likely to take place, where the emerged radicle was located. All treatments decreased the amount of pre-emergence blight. However, it is not recommended that plantings be made with chemicals in heavily infested soils, since the treatment does not prevent, but only retards the development of root rot. Treatment with Arasan in lightly infested soils may give good control.

Discussion

Some factor or factors must still be regarded as basic to the outbreak of 1945 and also that of 1949. It has been suggested that the newly introduced variety of asparagus, Viking (V-35), was more susceptible or that seed of it served as a carrier of the disease. However, under controlled conditions, Mary Washington was equally susceptible. Indeed, seedling beds of the latter were noted in 1949 to be diseased. Furthermore, it was not possible to isolate the pathogen from seeds of Viking or from seed washings. Conditions

which favored the disease may have existed in the soil environment during the spring of 1945. It has been demonstrated that temperatures between 15° and 20° C. favored pre-emergence blight while the optimum for post-emergence blight was established at 20°–30° C. It is entirely possible that the cold, wet spring of 1945 retarded the emergence of seedlings in newly seeded asparagus beds. Later in the season, a rapidly warming soil may have sprouted the seedlings and have favored a blight to the extent described by the growers.

It is apparent that the fungus must have been present in the soils concerned in the vicinity of the germinating seeds in sufficient quantity to start an outbreak of blight. Incidence of the pathogen in the soil, apart from the roots of the host, may be determined by the availability of food and by the ability of the fungus to utilize it. A particularly favorable substrate seems to be the decaying roots of mature asparagus plants, because outbreaks of blight in seedling plantations has been correlated with previous crops of asparagus.

Because of the diversity in the chemical composition of the plants grown and incorporated, it is difficult to attribute the effective increase in disease incidence in the soybeans, oats, red clover, and alfalfa series of continuous crops (Table VII), over that in the asparagus series, to the action of any specific substrate.

It must be pointed out that no parallel can be drawn between the build-up of the pathogen in a noninfested field soil and that observed during the course of the incorporation of these crops in somewhat rapid sequence. Had the natural sequence of cropping been simulated, only a single crop would have been grown in one year, and from six to nine months would have intervened between the time of incorporation of the residue and the replanting of asparagus. The significance of the physiological age and condition of the residues under field conditions and the colonization of them by microflora, during an intervening period of low temperature, has also not been considered.

In the environment of the soil, cellulose may be the most common source of carbon available to fungi. Within the host, unlignified cellulose, glucose, and plastic nitrogenous compounds are present. The pathogen must compete with other microorganisms in the soil, on the surface of the roots of the host, and even within the host. *F. oxysporum* var. *redolens* used sucrose more readily than *F. moniliforme*, while *F. moniliforme* broke down cellulose more rapidly than *F. oxysporum* var. *redolens*. Both organisms grew well on glucose while the *F. oxysporum* var. *redolens* hydrolyzed starch more easily than *F. moniliforme*. Both pathogens sought entry to the host through the root cap, which consists of a layer of dead cells probably composed mainly of cellulose. In the experiment with mixed inoculum of the two fungi (Table IV), the apparent reduction in the percentage of damped off seedlings suggests that the two were competing saprophytically for the substrate of the root cap. The seat of competition or antagonism may be the cellulose walls of the cells of the root cap. Within the roots, the amount of starch present, or the rate at which

it could be broken down into more labile carbohydrate, may be the limiting factor. It is suggested that the colonization of the root tips by *F. moniliforme* and the invasion of the cortical tissues by *F. oxysporum* var. *redolens* may be the result of their respective abilities to use cellulose and starch.

It may be pointed out here that specialization to certain parts of the host are encountered elsewhere in the genus *Fusarium*. *Fusarium orthoceras* Wall. var. *gladioli* McCull. is specialized in its parasitism of gladiolus to the vascular system and causes *Fusarium* yellows. However, some cortical decay may take place. Nelson (15) distinguishes a second fusarial disease of gladiolus, basal dry rot, caused by *Fusarium* sp. which affects the corms only at the first and second internodes. The major damage caused by this fungus is attributed to its destruction of the root-forming tissue. Forsberg (10) described *Fusarium* rot of gladiolus, caused by *F. oxysporum* (Schlecht.) var. *gladioli* Massey. He states that infections usually occur on the sides and lower halves of the corms. "Lesions are generally somewhat sunken with definite and slightly raised advancing margins. The active lesions show a narrow, water-soaked, dark-colored outer area, which blends sharply into the healthy tissue" (p. 71). This description certainly suggests a cortical rot similar to that caused by *F. oxysporum* var. *redolens* in asparagus roots.

The diversity of species of *Fusarium* attacking the cereals offers further examples of specialization. Sprague (19) reports *F. culmorum* (W. G. Sm.) Sacc. as causing root rot and head mold of wheat, *F. equiseti* (Corda) Sacc. as moderately parasitic on older seed plants, and *F. moniliforme* Sheldon as causing secondary root rot but only very mildly parasitic if introduced independently. Johnston and Greaney (12) found, in nature, isolates of *F. culmorum* (W. G. Sm.) Sacc., ranging from virulent parasites to virtual saprophytes, with some even less pathogenic than *F. equiseti* (Corda) Sacc. Therefore, it has been possible to find, within the same genus, and probably within the same species, individuals with parasitic capabilities ranging from those capable of initiating a disease independently by direct penetration to those which must follow the entry of others. One might add those which cannot invade a host but must confine themselves to a nonliving substrate.

Acknowledgments

The author wishes to express his gratitude to Dr. D. L. Bailey of the Department of Botany, University of Toronto, for suggesting this problem and for many helpful suggestions during the course of the investigation, and to Dr. G. H. Berkeley, Officer-in-charge of the Plant Pathology Laboratory, St. Catharines, Ontario, for making available the facilities at his disposal.

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EXPLANATION OF FIGS. 8-34

PLATE I

FIG. 8. Asparagus seedlings two months after emergence in soil artificially infested with *F. oxysporum* var. *redolens*.

FIG. 9. Crowns of seedlings six weeks old, showing injury typical of that caused by *F. moniliforme* Sheldon. × 2

FIG. 10. "Streak" lesion on six-week-old asparagus seedling root. × 2

FIG. 11. Six-week-old seedlings showing severe root rot after being grown in soil artificially infested with the pathogen. × ¾

FIG. 12. Secondary root of six-week-old asparagus seedling showing an elliptical lesion formed at the point of attachment of an infected lateral root. × 10

FIG. 13. Primary root of one-week-old asparagus seedling, showing a "sunken" water-soaked lesion. × 10

FIG. 14. Primary root of seedling infected by *F. oxysporum* var. *redolens*, slit open to show the completely disintegrated cortex and intact stele. × 10

PLATE II

FIG. 15. Cross section through "streak" lesion, stained in lactophenol and cotton-blue, showing an extensive layer of cork cells forming underneath the infected area. × 100

FIG. 16. Cross section of lesion showing chlamydospores formed in the parasitized cortical cells of a seedling which had become dried out. × 430

FIG. 17. Chlamydospore in cortical cell of asparagus seedling. × 970

FIG. 18. Cross section through lesion, stained in lactophenol and cotton-blue, showing intercellular hyphae. × 430

FIG. 19. Cross section, stained in lactophenol and cotton-blue, showing the progress of a hypha through a cell wall.

FIG. 20. Living unstained hyphae growing in water, from the edge of a lesion. × 430

FIG. 21. Epidermal cells of an infected root, with intracellular hyphae bearing appressoria at the penetration points indicated by arrows. × 970

FIG. 22. Intracellular hypha, with appressoria formed against a cell wall. × 970

FIG. 23. Intracellular hyphae in an epidermal cell of an infected seedling, mounted in water and photographed in the living condition without a stain. × 970

PLATE III

FIG. 24. Four-day-old single spore cultures of *F. oxysporum* var. *redolens*, on 2% potato-dextrose agar.

FIG. 25. Culture of *F. oxysporum* var. *redolens*, after one week of growth on 2% potato-dextrose agar, showing heads of spores produced near the point of inoculation. × 10

FIG. 26. Four-day-old single spore cultures on 2% potato-dextrose agar of fusaria proved or reported to be parasitic on asparagus. (two plates of each, showing upper and lower surfaces).

(1) *F. oxysporum* sensu Snyder & Hansen var. *redolens* (Wr.) Gordon
(2) *F. oxysporum* f. *asparagi* Cohen
(3) *F. moniliforme* Sheldon
(4) *F. culmorum* W. G. Sm.

FIG. 27. Macroconidia of *F. oxysporum* var. *redolens*, produced after one week of growth on 2% soil agar, and photographed in the living unstained condition. × 430

FIG. 28. Macroconidia of *F. oxysporum* var. *redolens*, produced on 2% soil agar, stained in lactophenol and cotton-blue and photographed through a yellow filter. × 430

FIG. 29. Microconidia of *F. moniliforme* Sheldon, produced on 2% soil agar. × 430

FIG. 30. Macroconidia of *F. moniliforme* Sheldon, produced on 2% soil agar. × 430

PLATE IV

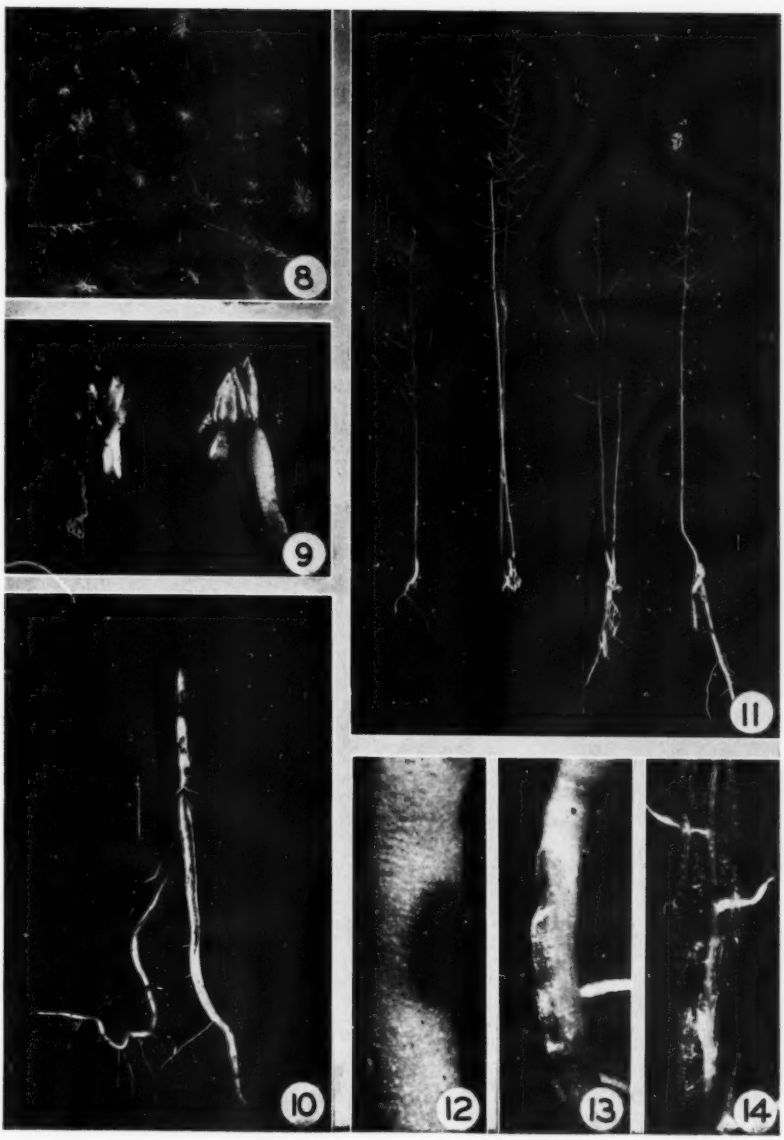
FIG. 31. Cultures of *F. oxysporum* var. *redolens*, after one week of growth on 2% potato-dextrose agar, at 25° C. (left) and 35° C. (right).

FIG. 32. Hyphae and spores of *F. oxysporum* var. *redolens*, after one week of development on 2% soil agar, stained in lactophenol and cotton-blue and photographed through a yellow filter. × 100

FIG. 33. Spores of *F. oxysporum* var. *redolens*, after one week of growth at 15° C. × 100

FIG. 34. Spores in the same culture as in Fig. 3, photographed two days after being shifted to 25° C. The area selected was at the time the shift was made. × 100

NOTE: Figs. 8–34 follow.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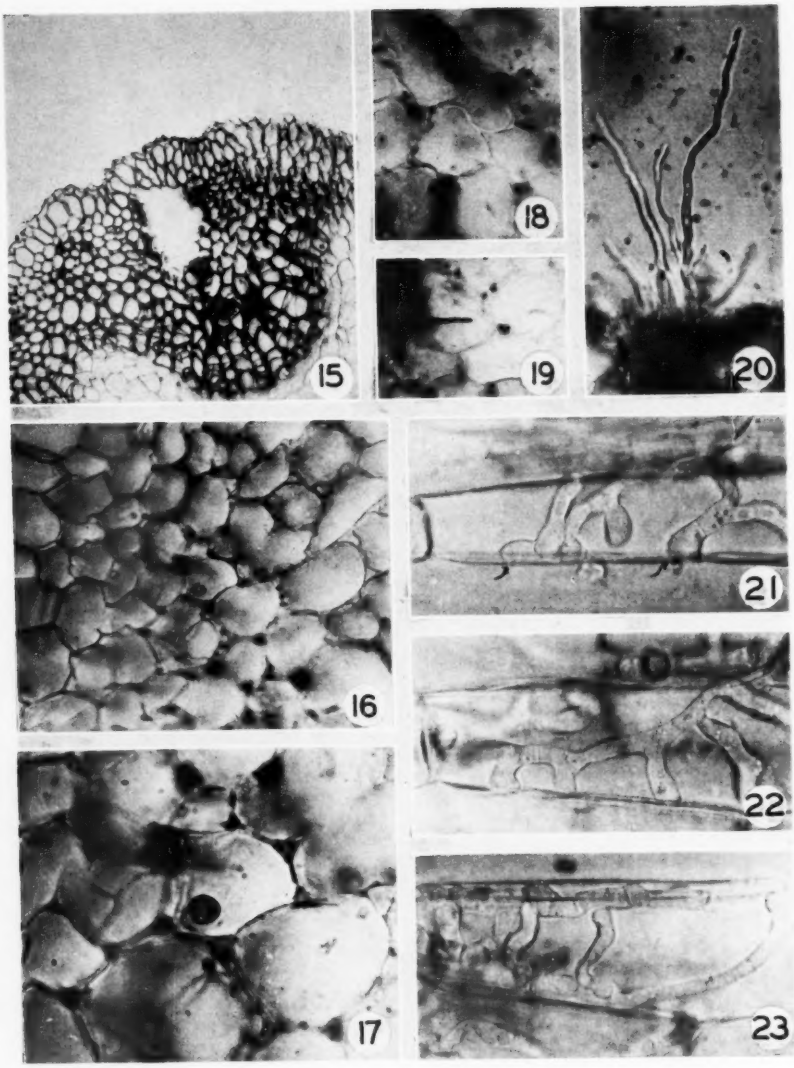
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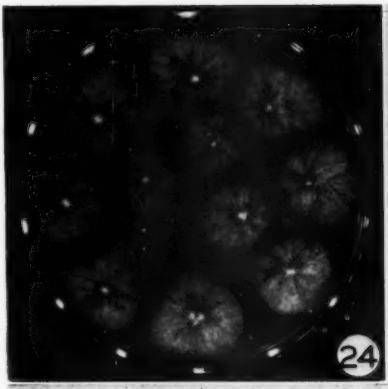
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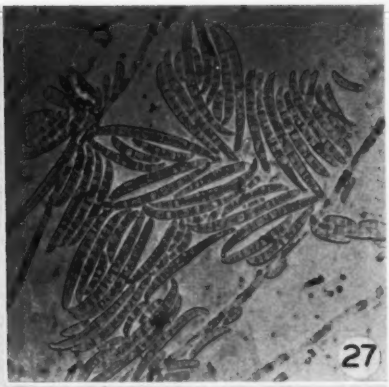
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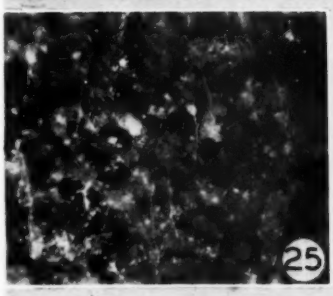
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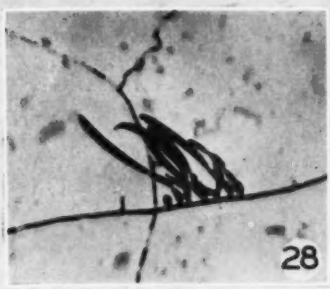
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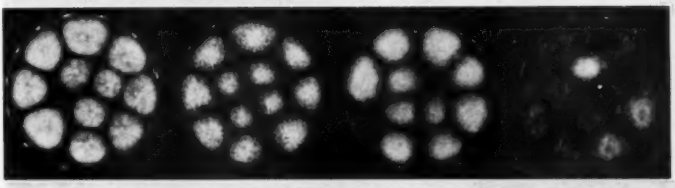

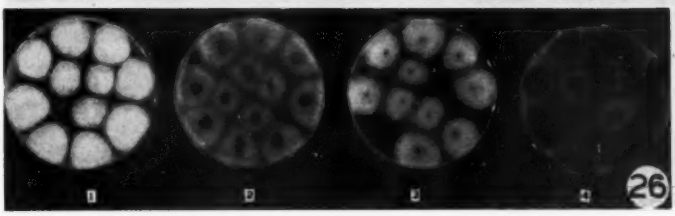
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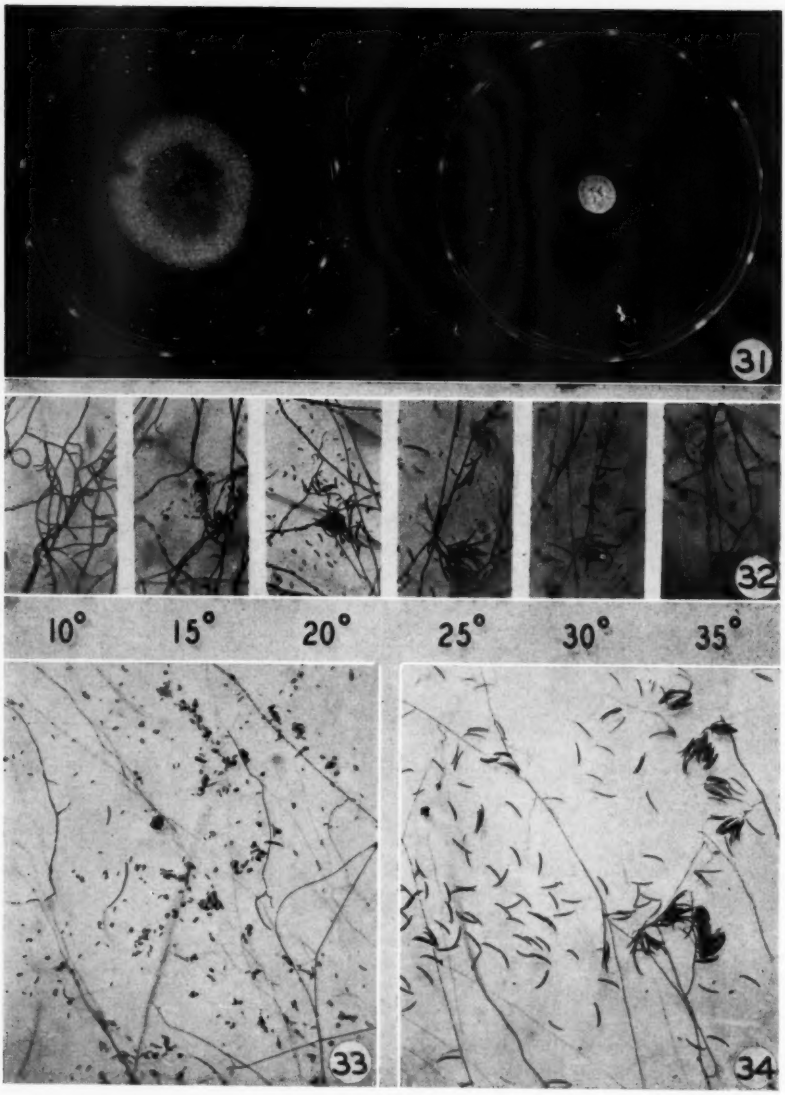
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ARCTIC AEROPALYNOLOGY

SPORA OBSERVED ON STICKY SLIDES EXPOSED IN VARIOUS REGIONS IN 1950[1]

By Nicholas Polunin

Abstract

Sticky slides were exposed more or less throughout the summer of 1950 in widely scattered arctic and other northern areas including Point Barrow, Alaska, northern-central Baffin Island, Jan Mayen Island, and Spitsbergen. Although the spora were generally sparse and conclusions must be regarded as tentative, it appeared that near ground level the incidence of pollen grains tended in general to coincide with the main period of flowering but that moss spores were in the air more or less throughout the growing season. Most (but by no means all) of the pollen observed could have been of local origin except in Spitsbergen, where there tended to be a preponderance of abietineous winged grains (mostly of *Pinus*) which were persistently caught practically throughout the brief summer although they had evidently been blown in from at least several hundred miles away.

Introduction

With the object of continuing and extending some aspects of the arctic aerobiological work already reported upon (14, 15, 16, 18, 19), particularly towards getting some idea of the pollen and spore dispersal picture in the North through the summer, arrangements were made for the exposure of sticky slides in Petri dishes in widely scattered arctic localities more or less throughout the season of 1950. This necessitated the generous co-operation or active assistance of the following gentlemen to whom warm gratitude is due: Dr. Ira L. Wiggins, then Scientific Director of the Arctic Research Laboratory, Point Barrow, Alaska; Lt. Col. P. D. Baird, then Director of the Arctic Institute of North America's Montreal Office, and his assistant (and now successor) Mr. Svenn Orvig; Mr. J. W. Burton of the Canadian Government Service, Ottawa; Dr. J. Warren Wilson, Department of Botany, University of Reading; Professor H. U. Sverdrup, Director of Norsk Polarinstitutt; Dr. Ivar Jørstad, Director of Statens Plantevern, Oslo, and several associates of the last two.

The main exposing was accomplished (1) near Point Barrow, Alaska, (2) on an ice-cap in northern-central Baffin Island, (3) on an icebreaker proceeding up Davis Strait and Smith Sound and northward to the north coast of Ellesmere Island around lat. 83° N., (4) on Jan Mayen Island off the east coast of Greenland, and (5) at Sarsbukta in West Spitsbergen. In addition the writer as organizer of the project made two northern Transatlantic flights at high altitudes with the hope of obtaining further evidence as to what aerobiota travel great distances, where they go, and what effect they have on the dissemination pattern. But owing to contamination of the controls, these attempts must unfortunately be written off as largely abortive. For

[1] *Manuscript received May 28, 1954, and as revised, May 16, 1955.*
Contribution from Harvard University, Cambridge, Mass., and Yale University, New Haven, Conn.

similar reasons the materials collected on the icebreaker must be treated with extreme reservation. In so far as any indications from these two attempts may be mentioned, they must not be allowed to enter into general discussion or form the basis of serious conclusions.

The accompanying sketch-map (Fig. 1) indicates the points or tracks of exposure in 1950. Each set of exposures will be considered separately below. Where possible, silicone-smeared slides stuck to the inner bottom surfaces of Petri dishes were supplied for the making of exposures, but in some cases necessity demanded the substitution of local expedients such as the use of Vaseline instead of silicone, or mounting on the spot instead of closure of the Petri dish for return to the laboratory for mounting. This laboratory mounting was done in a dust-free room using glycerine jelly containing basic fuchsin made up as prescribed by Dr. Roger P. Wodehouse. In all material except that collected on the Transatlantic flights and on the icebreaker, control slides indicated cleanliness of the materials and validity of the results. In view of the later contentions of Gregory (7, 8, 9), it seems possible that the catching might have been more effective if the slides had not been exposed face-upwards

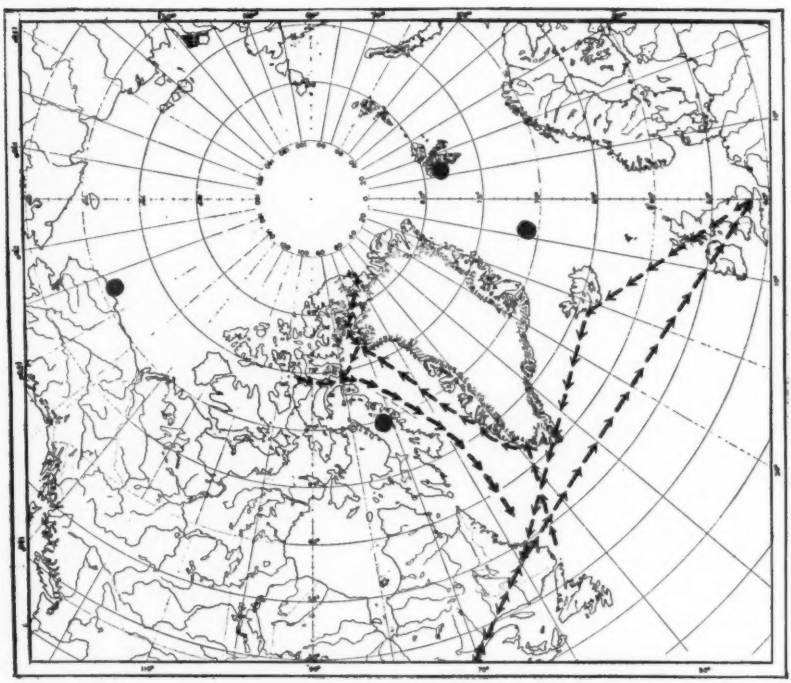

Fig. 1.

in the conventional manner. Where necessary, attempts were made to screen the slides from wetting, but these were not always successful.

Although fungal spores were frequently observed on the exposed slides, and instances are mentioned at the foot of Tables II and III, no attempt was made to record them systematically.

Exposures

1. Near Point Barrow, Alaska

The exposures were made near sea-level "on a tower about 20 feet above the ground and situated a bit over 100 yards from the street that runs through camp" (I. L. Wiggins *in litt. ann.* 1950) which lies in about lat. 71° 20′ N. and long. 156° 46′ W. a few miles to the south-west of the northernmost tip of Alaska. On one occasion (the exposure of August 22-23) both of the slides were blown out of the Petri dish and lost, but apart from that, the exposures ran continuously from June 28 to September 4, each being for a period of about 24 hr. On each slide an area of approximately 50 × 24 mm. was examined microscopically; the results are indicated in Table I.

From these results it seems clear that, at least in this area in 1950, there was little pollen or bryophytic spore material in the air from June 28 to August 2 (20 such air "spora" (9) on 35 slides), whereas from August 2 to the termination of observations on September 5 there was relatively much (70 examples on 33 slides). According to Dr. Wiggins (*in litt. ann.* 1955), in this region in 1950 "the main peak of the blooming was by no means over at the end of July. In fact, the ice did not go out until July 6th, and we collected comparatively few plants until about the 20th of that month, and the good collecting came in late July and early August. I suspect that the high percentage of pollen late in the season and little earlier was a reasonably accurate reflection of the phenology of that year." With regard to moss spores, Dr. William C. Steere remarks (*in litt. ann.* 1955), following experience in the same region, that these "are probably liberated throughout the season in the Arctic. The capsules mature fairly late in the season, that is, in late July, throughout August, and into early September. However, spores remain in the capsules through the winter even though they are gradually released through the activity of wind, rain, etc. Since the spores are quite resistant, there is no biological reason why they should not continue to be distributed...." Although the lateness of the season of most of the pollen, particularly, had at first made it seem likely that the grains tended to persist long after their liberation, for example by being blown up again and again after settling, the above considerations appear adequate to explain the distribution in time which was observed for them. As for provenance, it may be noted that the vast majority of significant objects appeared to be local, or at least northern Alaskan, while there was no certainty in any instance of a more distant origin than northern-central Alaska a very few hundred miles away.

TABLE I

POINT BARROW, ALASKA

Dates	Total pollen grains and moss spores	Grasses	Cyperaceae	*Salix*	Populoid	*Betula*	*Alnus*	Rumicoid	Ericales	Compositae	Juniperoid	Undet. p. gr.	Prob. moss spores
June 28-July 5 (7 slides)	0												
July 5-6	2											2	
6- 8 (2 sl.)	0												
8- 9	1				1								
9-10	1												1
10-14 (4 sl.)	0												
14-15	1											1?	
15-16	0												
16-17	3	1											2
17-18	2			1?									1
18-21 (3 sl.)	0												
21-22	3					1?		1					1
22-23	3		2									1	
23-24	1		1										
24-25	0												
25-26	1											1	
26-28 (2 sl.)	0												
28-29	1				1?								
29-30	1											1	

TABLE I—*Concluded*

POINT BARROW, ALASKA—*Concluded*

Dates	Total pollen grains and moss spores	Grasses	Cyperaceae	*Salix*	Populoid	*Betula*	*Alnus*	Rumicoid	Ericales	Compositae	Juniperoid	Undet. p. gr.	Prob. moss spores
30-Aug. 2 (3 sl.)	0												
Aug. 2-3	1											1	
3- 4	3	1											2
4- 5	4	3	1										
5- 6	0												
6- 7	3	1	1										1
7- 8	3				1				1				1
8- 9	6	3			2								1
9-10	1	1											
10-11	1												1
11-12	1										1		
12-14 (2 sl.)	0												
14-15	1					1?							
15-16	0												
16-17	1											1	
17-18	7	1								1			5
18-19	4			1?			1?						2
19-20	1	1											
20-21	1												1
21-22	5	1			1		1?					2	
23-24	7	1	1?			1						3	1
24-25	3	3											
25-26	2	1											1
26-27	0												
27-28	2												
28-29	4											1	3
29-30	1												1
30-31	1												1
31-Sept. 1	3												3
Sept. 1-2	0												
2- 3	1	1											
3- 4	3											1	2
4- 5	1	1											

TABLE II

BAFFIN ISLAND

Dates	Total pollen grains and moss spores	Grasses	Cyperaceae	*Salix*	Populoid	*Betula*	*Alnus*	Rumicoid	Caryophyll	Ericales	Compositae	Juniperoid	Undet. p. gr.	Prob. moss spores
June 10-11	0													
14-15	2					1							1	
15-16	0													
16-17	2					1	1							
17-20 (3 sl.)	0													
20-21	2									1			1	
21-23 (2 sl.)	0													
23-24	2	1									1			
24-27 (3 sl.)	0													
27-28	1		1											
28-29	1						1?							
29-30	1									1?				
30-July 1	0													
July 1-2	1									1				
2- 3	0													
4- 5	1												1	
5- 9 (3 sl.)	0													
9-10	2									1				1
10-11	0													
11-12	1			1										
12-13	1					1								
13-14	0													
14-16	1												1	
16-17	1													1

TABLE II—*Concluded*

BAFFIN ISLAND—*Concluded*

Dates	Total pollen grains and moss spores	Grasses	Cyperaceae	*Salix*	Populoid	*Betula*	*Alnus*	Rumicoid	Caryophyll	Ericales	Compositae	Juniperoid	Undet. p. gr.	Prob. moss spores
17-18	10		1							1				8
18-19	2					1							1	
19-20	1									1				
20-21	2									1			1	
21-22	1		1											
22-23	1		1											
23-24	1						1?							
24-27	3							1		1			1	
27-28	5						1	3						1
28-29	8				2	2?		2					1	1
29-30	6		1				2						3	
30-31	1						1							
31-Aug. 1	4	1				2?							1	
Aug. 1-2	1													1
2- 3	1					1								
3- 4	3							3						
4- 5	1												1	
9-11	3					1?	1?		1					
11-12	0													
15-16	4					1					2			1
17-18	3										1		1	1
22-23	0													
23-24	1													1
25-26	1											1		

In addition, fungal spores were observed on several of the slides—e.g. nine spores of Ustilago on August 3–4, one spore of Alternaria on August 22–23, and one spore of Ustilago on August 25–26.

2. On an Ice-cap in Northern Baffin Island, at Approximately 69° 42′ N. and 72° 13′ W.

The exposures were made on top of a meteorological tower 23 ft. above the surface and at a total altitude of about 2860 ft. above sea level (P. D. Baird *in litt. ann.* 1950). The tower was situated some nine miles from the margin of the ice-cap (*ibid. ann.* 1952) and consequently about that distance from the nearest terrestrial plants. In this instance exposing commenced on June 10th and terminated on August 26th, but there were several gaps of up to four days and nights at a time, and, although the vast majority of the exposures were of approximately 24-hr. duration, some were of less than half that period and others, again, were up to three times as long. The area microscopically examined on each slide in this set was approximately 50 × 22 mm.; the results are indicated in Table II.

In this case again it seems that there was much more pollen and allied material in the air later on than there was early in the summer. Thus, excluding fungal spores which were not systematically reported, there were observed, on 31 slides exposed up to midday on July 17 for a total of approximately 765 hr., 19 pollen grains and probable moss spores, and, on 24 slides exposed thereafter and up to August 26 for the much smaller total of approximately 631 hr., no less than 63 such spora. According to the separate testimonies of Drs. Pierre Dansereau and Mason E. Hale (*in litteris binis ann.* 1955), who were the botanists of the party, most of the flowering was in July and early August in this region in 1950. But in view of the statement of Dr. Dansereau that "the peak of flowering for the majority of species did not precede July 17th by very much and flowering was by no means over at that time, although it had decreased enormously by August 1st", it is difficult not to retain the impression already gained (17) that there is some tendency for pollen near the ground to remain more plentiful in continental arctic regions than in insular ones as the season advances.

Most of the slides bearing more than a single significant object from the first half of the period of observation had been exposed to winds coming from the east. Moreover, there was not determined on any of the slides of this set, whether exposed early or late, a single pollen grain that could not have come from West Greenland or the northern parts of the Labrador–Ungava Peninsula less than a thousand miles distant. Indeed only the *Alnus*, populoid, and juniperoid grains were necessarily of other than local origin; *Alnus* and *Juniperus* occur in southwestern Greenland (the latter northward almost to the latitude of these exposures) and both they and *Populus* are plentiful and widespread in northern Ungava–Labrador and the mainland of the Canadian Northwest Territories farther west.

3. Davis Strait and Northward to ca. 83° N.

The exposures on the icebreaker voyage northward to the north coast of Ellesmere Island were made with the help of a holder built ahead of the forward mast and about 50 ft. above the surface of the water (J. W. Burton

voce). Most unfortunately the results obtained from this material ought probably to be discarded on account of contamination of the controls; at best, they cannot be considered reliable. However, in view of the lesson learned (15, 19) more than fourteen years after I had discarded my first high-latitude exposures in Lapland and Spitsbergen in 1933, it seems appropriate to mention that nearly one-third of the slides exposed on this icebreaker voyage (mostly in August) bore pollen grains of *Ambrosia* and nearly one-fifth bore winged pollen grains of Abietineae, while two-fifths bore pollen grains of *Betula*. This was a higher incidence than was observed on any of the contaminated controls or unexposed residual slides and so *may* be significant, though in view also of the looseness of many of the slides in the Petri dishes and partial breaking of some of the latter it must be emphasized that no serious account should be taken of these 'observations' without properly controlled repetition.

4. Jan Mayen Island, at Approximately Lat. 71° N. and Long. 8° 40′ W.

As usual the slides were exposed in Petri dishes, "on the top of a post 1½ metres above ground level, under a metal cover as figured by Wodehouse. The exposure point was 20 m. above sea level, on a hillside in the central waist of the island . . . Slides were exposed for consecutive three-day periods, replacements being made at approximately 10.00 a.m. (G.M.T.)" (J. W. Wilson *in litt. ann.* 1950). Strong winds blew away and lost the first two sets to be exposed, and the mists and light rains were so frequent that "the slides were commonly wet even under the cover." Effective exposures extended from June 28 to August 21. In this set an area of approximately 38 × 19 mm. was microscopically examined for each of the 72-hr. exposures; the results are indicated in Table III.

TABLE III

JAN MAYEN ISLAND

Dates	Total pollen grains and spores	Grasses	*Salix*	*Betula*	Rumicoid	Unident. pollen grain	Fern spore	Prob. moss spores
June 28-July 1	0							
July 1-4	1				1			
4- 7	1							1 (tetrad)
7-10	7				2			5
10-13	6				6			
13-16	5	3			2			
16-19	1	1						
19-22	1	1						
22-25	2		1	1				
25-28	1	1						
28-31	1				1			
31-Aug. 3	6	4			2			
Aug. 3-6	1							1
6- 9	5	5						
9-12	1						1	
12-15	3	3						
15-18	1					1		
18-21	0							

In addition, fungal etc. spores were observed on several of the slides, including 14 apparently of a Myxomycete (July 19–22) and five of Cladosporium (July 22–25).

Apart from suggestions of a lag in early summer (two observed spora on the first three slides) and reduction toward the end (one such body on the last two slides), no marked seasonal variation was noted in this set. Nor was such to be expected in view of the isolated position of Jan Mayen Island, the protracted flowering-season of many of its plants, and the fact that, apparently, almost all of the spora observed could have been, and probably were, of local origin. Thus *Oxyria* is said to be about the commonest flowering plant on the island (J. Lid *voce*), and the Gramineae are represented by more species than any other family in its known vascular flora, among which a single fern, *Cystopteris fragilis* s.l., occurs. Whether the local material belongs to the rugose- or spiny-spored form, or both, of *Cystopteris fragilis* s.l., does not appear to have been determined, but the spore in question corresponded to the former, which is the more widespread on the adjacent coast of Greenland, where, however, both occur (12). The only other pollen grains determined from this material were a single one of *Salix* (*S. herbacea* is one of the commonest plants on the island) and one of *Betula*, which is not known to occur on Jan Mayen Island but is plentiful in the adjacent parts of Iceland and East Greenland only a very few hundred miles distant.

5. Sarsbukta, West Spitsbergen

The point of exposure lies in about lat. 78° 44′ N. and long. 11° 43′ E. on the east (main island) shore of Forlandsundet, where in 1950 "Unfortunately the weather was generally bad, and in many instances it was impossible to prevent rain falling on the slides" (H. U. Sverdrup *in litt. ann.* 1950). The exposures ran from July 17 to August 22 (with one gap from July 21 to 22) and were made "abt. 20 m. above sea level. The dishes were placed on a stake abt. 2 feet above ground. Abt. 10 cm. above the dish was placed a wooden plate" in an attempt to shelter the slides from rain. "The place of exposure is a wide plain covered with gravel and other loose material, where much of the flora characteristic of Svalbard is found. All dishes were exposed from abt. 8 p.m. until the same hour the following day" (Anders K. Orvin *in litt. ann.* 1951). In this set the area microscopically examined each time lay under a 50 × 24 mm. cover slip but owing to uneven mounting with the smear used the actual area reported upon varied from an estimated half to virtually the whole of this area. On this occasion fungal spores were entirely ignored. Results are indicated in Table IV.

As in the observations made on the rather similarly isolated Jan Mayen Island there is no very marked seasonal variation to be noted in this set of observations, though the data are not numerous enough to allow of more than purely tentative conclusions. However, the first 12 exposures (July 17-30) in this high-arctic land gave a total of 21 significant objects, and the last 13 (August 9-22) gave 14, which, with the 'gap' beginning on July 31, suggests a falling off in pollen incidence with flowering. This is accentuated by subtraction of the abietineous winged pollen grains which must have come from afar, leaving 13 for the first 12 exposures and only 6 for the last 13. These

TABLE IV

WEST SPITSBERGEN

Dates	Total pollen grains and moss spores	Grasses	*Salix*	*Betula*	Rumicoid	*Ulmus*	*Picea*	*Pinus*	Unident. p. gr. or moss spores
July 17-18	2						1		1
18-19	2	1							1
19-20	1							1	
20-21	2				1				1
22-23	2				1			1	
23-24	2				2				
24-25	3							2	1
25-26	0								
26-27	2								2
27-28	1								1
28-29	1				1?				
29-30	3			1				2	
30-Aug. 5 (6 sl.)	0								
Aug. 5-6	3							2	1
6- 7	1			1					
7- 8	1								1
8- 9	0								
9-10	1	1?							
10-11	1		1						
11-12	4				1?			3	
12-13	1							1	
13-14	1							1	
14-15	1							1	
15-17 (2 slides)	0								
17-18	3					1		1	1
18-19	0								
19-20	1								1
20-21	0								
21-22	1						1		

and the following points, however, with the meager material available and rather fortuitous sampling accomplished, are largely supposition at this stage. Apart from the single *Ulmus* pollen grain which was found on a slide in a container so badly broken that it was noted from the time of mounting as probably best ignored, all the non-abietineous material could well be, and probably was, of local origin. On the other hand the relatively plentiful importation of abietineous pollen from, with little doubt, the vast coniferous forests of which the nearest outliers start about a thousand kilometers to the south (and much farther away to the east and west) is evident and highly significant, and confirms and extends Wille's early suggestion (21) concerning his observation of pollen of *Pinus* among freshwater algae collected well north in Novaya Zemlya by Kjellman. The protracted period of pollen occurrence, especially of these large and buoyant grains seems likely to be due to frequent sedimentation or removal from the air with precipitation, and subsequent blowing up again with changes of wind velocity or direction. Even though most of the air-borne particles of this nature that are disseminated by a plant may tend to come to rest in the general vicinity of the plant (1), it is now well known that pollen grains, e.g. of various Coniferae and Amentiferae, can be dispersed by winds over great distances (2, 4, 5), the longest known distance being apparently that travelled by pollen of *Nothofagus* in the southern hemisphere where pollen transport of up to 4500 km. has been claimed (10). Consequently this Spitsbergen observation is not particularly surprising, especially in the light of Elton's earlier report (3) of living aphids etc. being blown in considerable numbers to the same archipelago apparently from the spruce forests of Kola Peninsula nearly 1400 km. away (6). What is surprising, and disturbing in its implications, is the apparent preponderance through much of the summer of an exotic pollen blown in from a very considerable distance!

According to information kindly furnished by Professor H. U. Sverdrup (*in litt. ann.* 1954), the flowering of *Pinus* in northernmost Scandinavia in 1950 extended from June 18 to July 10 "and in no circumstances to beyond July 20." In the latter half of June, several stations in this region reported frequent cumulus clouds and, inland, some cumulo-nimbus, indicating "a lively vertical convection which may have brought pollen to great altitudes." At the same time there developed, and persisted especially during the last week of June, a northward-moving flow of air at high altitudes (500 millibars and above) between northernmost Scandinavia and Spitsbergen. This flow was weak over the land but over the sea reached a velocity of 30 knots or more, and may well have carried clouds of pollen to Spitsbergen; on the other hand the period of July 1–20 was "characterized by a high pressure area over the Norwegian Sea and a transport of pollen can hardly have taken place during that period."

In view of the notorious durability of many pollen grains it thus seems that a single heavy (or prolonged lighter) 'rain', in this case of *Pinus* pollen arriving early in the summer, may, after grounding, for example in aqueous precipitation, act as a reservoir for future weeks or even months during which grains

are apt to be blown up into the air again. The very scantiness but protracted nature of the records in 1950 suggested that these grains were far from their source (1). Whether or not grains would remain viable in such circumstances is open to conjecture. But in view of the prolonged viability which pollen of some mesothermic or even tropical plants can exhibit under suitable conditions, it seems quite likely that some grains would retain their powers of germination, and consequently conceivable that such long-distance pollination and hence "absent-treatment" hybridization may occur (15, 16). There are numerous known instances of pollen being preserved apparently unharmed for more than a year, low temperatures being particularly effective in this connection, and according to one recent report (20) pollen of *Ginkgo* can be kept for over two years with only slightly reduced powers of germination.

It was hoped that the two northern Transatlantic flights undertaken by the writer for quantitative sampling of the atmosphere at high altitudes and different times during the season of exposure would give some indication of the source and distribution of air spora and, especially in relation to the computed origins and trajectories of the air-masses involved, help complete the spore-dispersal picture in the North in summer. For this purpose an electrostatic bacterial air sampler was employed as described earlier (11). Most unfortunately, however, unexplained contamination of several of the controls makes it necessary to treat these observations with extreme caution, as in the case of the material collected on the icebreaker voyage (No. 3, above). The sticky slides were prepared in a laboratory other than the writer's, and on these occasions without his supervision. All that can be stated, very tentatively, is that there appears to have been a considerable range though sparse 'population' of pollen grains even in mid-Atlantic between 8000 and 10,000 ft. in mid-June and early August, and that east-moving air-masses of long duration may have transported pollen grains of American plants such as *Ambrosia* and *Carya* to a position over the British Isles, while north- and then west-moving air-masses from the vicinity of the latter appeared to be carrying pollen of *Fraxinus* and *Ulmus* as well as the more usual *Betula*, grasses, and winged grains of Abietineae to the vicinity of Iceland. For the reasons already stated it must be emphasized that these are the merest suggestions on which nothing should be based without further, valid observation using properly prepared and tested materials and techniques, though it may be noted that Pady and Kelly (13) have recently reported that considerable numbers of viable fungal spores and bacteria occur over the North Atlantic Ocean especially in tropical air.

Tentative Conclusions

It will be seen from the above that, quite apart from the two sets of observations which are insufficiently secure to be brought into discussion, the data are such that any general conclusions must be reserved as tentative. This is the case *inter alia* because the observations are relatively few and limited, and moreover refer only to a single summer. They may, however, be valuable as pointers to future work, and, as such, seem worthy of record here.

With the reservation that any conclusions should be considered at this stage as no more than reasonable speculations, it may be said that the picture which seems to be emerging is of mainly short-distance dispersal of locally produced spora near land-level in the Arctic in summer, supplemented by some relatively long-distance dispersal in such special instances as *Pinus* pollen to Spitsbergen. Whether there is frequent still longer distance dispersal of the more buoyant pollens at high altitudes, for example where the winds are of great velocity and there is less likelihood of removal by precipitation, remains to be determined with certainty, owing to the need to omit from serious consideration the observations made on the Transatlantic flights and icebreaker voyage. With the exception of those of *Pinus* in Spitsbergen, only rather few odd pollen grains of obviously distant origin were observed on land in the material collected in 1950. The incidence of locally produced pollen grains appears largely to coincide with the most active period of flowering.

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COMPARATIVE STUDIES OF NORTH AMERICAN AND EUROPEAN CULTURES OF THE ROOT ROT FUNGUS, *FOMES ANNOSUS* (FR.) COOKE[1]

By D. E. Etheridge[2]

Abstract

Cultures of *Fomes annosus* originating in Europe could not be distinguished from those originating in North America either by colony appearance, growth rate, pH optimum, or cellulolytic activity. Three growth rate types on 2.5% malt agar were recognized and these are ascribed to individual variation rather than to host or geographical influences. Successive subculturing produced variants that fell into three growth classes. Half of the isolates displayed spontaneous, but reversible, changes in growth rate and colony appearance during subculturing and this is discussed from the standpoint of genetical and environmental influences. Cultures displaying different morphological characteristics and linear-growth rates differed little metabolically; each had a similar pH optimum ranging from 4.6 to 5.5, and each proved capable of altering the initial acidity of the medium to a reaction which was more suitable for growth. Two cultures were characterized by double pH optima at 4.6 and 5.5. Cultures having different linear-growth rates produced about the same dry-weight of mycelium on a cellulose substrate in a semisynthetic nutrient solution. On the basis of a statistical analysis of cellulose utilization by representative isolates it was impossible to distinguish between North American and European cultures.

Introduction

Judging from the number of recent reports dealing with the occurrence of *Fomes annosus* (Fr.) Cooke in North America, this fungus is becoming increasingly prominent as the cause of root rot in numerous native coniferous hosts and the disease threatens to become as serious as it has been in Europe for over a century. Although the fungus frequently has been found associated with the death of immature trees in the older established plantations of the United States (5, 12, 22, 23), attacks have been recently reported in pure or nearly pure naturally regenerated stands in the western white and ponderosa pine types in California (15, 24), and it is held responsible for major decay losses in mature western white pine (7, 9) and western hemlock (1, 6, 18). The increasing prominence of *F. annosus* in North America, particularly in plantations, has been ascribed by some investigators to changing trends in silvicultural and planting practices, leading inevitably to conditions which are known to favor the disease in Europe. Artificially altered environmental conditions, however, cannot be regarded as the only reason for the growing importance of the fungus since the presence of the disease has been reported in naturally reproduced stands of native mixtures, which involve few or none of the factors associated with unfavorable cultural and cutting practices. While many attempts have been made to study the various influences of site, largely by European investigators, notably Peace (14) and Rishbeth (19, 20) in England, and Rennerfelt in Sweden (16, 17), relatively little attention has been

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Contribution No. 176, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Assistant Forest Pathologist, Laboratory of Forest Biology, Calgary, Alberta.*

directed to the possible influence of distinct physiological races of the fungus upon variations in the severity of the disease in North America and abroad. Roll-Hansen (21) has furnished convincing evidence indicating an absence of distinct strains of *F. annosus* among isolates from Eastern and Western Norway, but his data do not provide a satisfactory basis for assessing the nature of strain variability over a wide geographic range. It was felt that information on North American and European isolates of this fungus, comparable in nature to that obtained by Roll-Hansen for isolates in Norway, would have more general application to root rot disease problems on both continents.

A comparison of growth and variability under uniform conditions of cultures *in vitro* of *F. annosus* originating from various localities in North America and Europe should furnish some clue as to whether distinct physiological races of this fungus occur in nature. An approach to this problem, through studying the occurrence of variants arising from subculturing, the effect of hydrogen-ion concentration in malt agar on the radial growth rate, and the effect of hydrogen-ion concentration on the cellulolytic and growth activity of representative European and North American isolates, has been made.

Materials and Methods

Sixteen cultures of *F. annosus* originating from various hosts and localities in North America and Europe were obtained for the purpose of conducting comparative studies. The cultures within each geographic group were not strictly similar in age or history, but were comparable in the sense that isolates of approximately the same age occurred indiscriminately in both groups. Data pertaining to the cultures are presented in Table I. The original culture number is shown in brackets, and the numbers used in this work are accompanied by either the letter *A* (North America) or *E* (Europe), the letter depending upon the origin of the culture.

Subcultures were made by transferring a suspension of conidia in a 3-mm. nichrome loop to the new substrate. For comparative studies of *F. annosus* in a liquid culture a standard inoculum was prepared from three-week-old plate cultures by pouring 10 ml. of sterile distilled water over the sporulating surface of the culture mat and collecting, after some agitation, a dense concentration of spores (conidia) in a Beckman vial. The optical density of each spore suspension was determined on a Beckman spectrophotometer, and each spore suspension was brought to a comparable level by the addition, if necessary, of more water. Spore counts, previously made using a haemocytometer and related to optical density values, provided a basis for estimating the number of spores per milliliter of suspension. Flasks were inoculated with 1 ml. (containing about 5.5×10^5 spores) of the standardized suspension by means of a sterile calibrated pipette. Wide-necked (6-cm.) 8-oz. jars equipped with a screw-top lid and containing 30 ml. of nutrient solution were used. The basal medium contained (per liter) $MgSO_4 \cdot 7H_2O$ (Merck) 0.75 gm.; asparagin (Brickman) 5 gm.; thiamine hydrochloride (Eastman) 0.03 gm. Phosphates and potassium were supplied in a buffer by adding varying

TABLE I

SOURCE OF CULTURES

Accession No.	Herbarium	Collection particulars	Isolation particulars
A-1 (8429)	Mycological Herbarium, Dept. Agr., Ottawa. (M. K. Nobles)	By I. Mounce and W. Touzeau on *Pseudotsuga taxifolia* (?) B.C., Canada. Sept. 17, 1938	From sporophore
A-2 (9326)	Mycological Herbarium, Dept. Agr., Ottawa. (M. K. Nobles)	By J. E. Bier on *Tsuga heterophylla* B.C., Canada	By I. Mounce
A-3 (8)	Osborn Botanical Laboratory, Yale University. (H. McNabb Jr.)	By W. A. Campbell on *Juniperus virginiana* Union, S.C., U.S.A.	By W. A. Campbell, from sporophore, Sept., 1949.
E-4 (100P)	For. Comm., For. Res. Stn. Alice Holt Lodge, Eng. (T. R. Peace)	By T. R. Peace on *Pinus sylvestris* Surrey, England. Oct. 9, 1951	By Batko, from sporophore (?), Nov. 21, 1951
E-5 (22426) (242S)	Mycological Herbarium, Dept. Agr., Ottawa. (M. K. Nobles)	By F. Roll-Hansen on *Vaccinium myrtillus* Eastern Norway	By F. Roll-Hansen, from root tissue, Sept. 1, 1939
E-6 (20bS)	Norges Landbrukshogskole, Botanisk Institutt. Vollebekk, Norway. (F. Roll-Hansen)	By F. Roll-Hansen *Pinus sylvestris* Vest-Agder, Fjaere, Norway	By F. Roll-Hansen, from sporophore, July 17, 1937
E-7 (F1288)	Mycological Herbarium, Dept. Agr., Ottawa. (M. K. Nobles)	By J. Liese on pine or spruce, Eberswalde, Germany	From sporophore
E-8 (9260) (2696)	Mycological Harbarium, Dept. Agr., Ottawa. (M. K. Nobles)	By T. R. Peace on *Larix* sp.	R. Macrae, July 12, 1939
E-9 (22427)	Mycological Herbarium, Dept. Agr., Ottawa. (M. K. Nobles)	By F. Roll-Hansen on *Calluna vulgaris*	By F. Roll-Hansen, from tissue
E-10 (53cS)	Norges Landbrukshogskole, Botanisk Institutt, Vollebekk, Norway. (F. Roll-Hansen)	By F. Roll-Hansen on *Calluna vulgaris* Western Norway	By F. Roll-Hansen, from wood tissue in the lower part of stem, Sept. 8, 1937.
E-11 (98bS)	Norges Landbrukshogskole, Botanisk Institutt, Vollebekk, Norway. (F. Roll-Hansen)	By F. Roll-Hansen on *Sorbus aucuparia* Western Norway	By F. Roll-Hansen, from sporophore, Sept. 30, 1937
E-12 (121aS)	Norges Landbrukshogskole, Botanisk Institutt, Vollebekk, Norway. (F. Roll-Hansen)	By F. Roll-Hansen on *Betula* sp. Eastern Norway	By F. Roll Hansen, from sporophore, Nov. 11, 1937
E-13 (242S)	Norges Landbrukshogskole, Botanisk Institutt. Vollebekk, Norway. (F. Roll-Hansen)	By F. Roll-Hansen on *Vaccinium myrtillus* Eastern Norway	By F. Roll-Hansen, from root tissue, Sept. 1, 1939
E-14 (1090/3)	Norges Landbrukshogskole, Botanisk Institutt. Vollebekk, Norway. (F. Roll-Hansen)	By F. Roll-Hansen on *Pinus sylvestris* Eastern Norway	By F. Roll-Hansen, from sporophore, June 25, 1951
E-15 (1082/2)	Norges Landbrukshogskole, Botanisk Institutt. Vollebekk, Norway. (F. Roll-Hansen)	By F. Roll-Hansen on *Picea abies* Eastern Norway	By F. Roll-Hansen, from sporophore, June 25, 1951
A-16 (83A)	Osborn Botanical Laboratory, Yale University. U.S.A. (H. McNabb, Jr.)	By David Smith on *Pinus resinosa* New Haven, Conn., U.S.A. Jan. 1951	By H. S. McNabb, from sporophore, Feb. 1, 1951

amounts of sterile $M/3$ K_3PO_4, $M/3$ KH_2PO_4, and $M/3$ K_2HPO_4 to the medium after sterilization. Twenty per cent of the final medium was buffered to obtain a range of reaction of pH 3.0 to 7.0. Carbohydrate was supplied in the form of glucose or cellulose, the cellulose was in the form of filter paper (Whatman No. 40, 5.5 cm.) and introduced into the nutrient basal solution by adding one filter paper to each culture jar. Sterilization was done at 18 p.s.i. for 10 min. After inoculation, the culture jars were incubated at 23° and 25° C. for 17 days. Cultural studies on a solid medium were made in 10-cm. Petri dishes containing 20 ml. of 2.5% malt agar, with or without the addition of buffer. Growth was determined by measurement of the colony diameter at the end of the seventh day. The pH of the solid medium was determined colorimetrically by crushing some of the agar with sulphonephthalein indicators and by comparing the color reaction with the

color equivalents given by Clark (4). The pH determinations were checked from time to time electrometrically using a glass electrode in diluted agar.

To provide quantitative data on the amount of cellulose decomposed in the presence of mycelium the following procedure was adopted. The amount of cellulose utilized by the test organisms was determined by calculating the loss-in-weight of the partially degraded filter paper according to the formula:

$$\text{cellulose utilized} = C_1 - (C_2 - MM) .$$

C_1 and C_2 were the oven-dry-weights of the filter paper before and after incubation with the fungus. MM was the oven-dry-weight of the mycelial fraction of the degraded filter paper read from a standard nitrogen–mycelial dry-weight curve (Fig. 1) prepared from results obtained using a glucose–

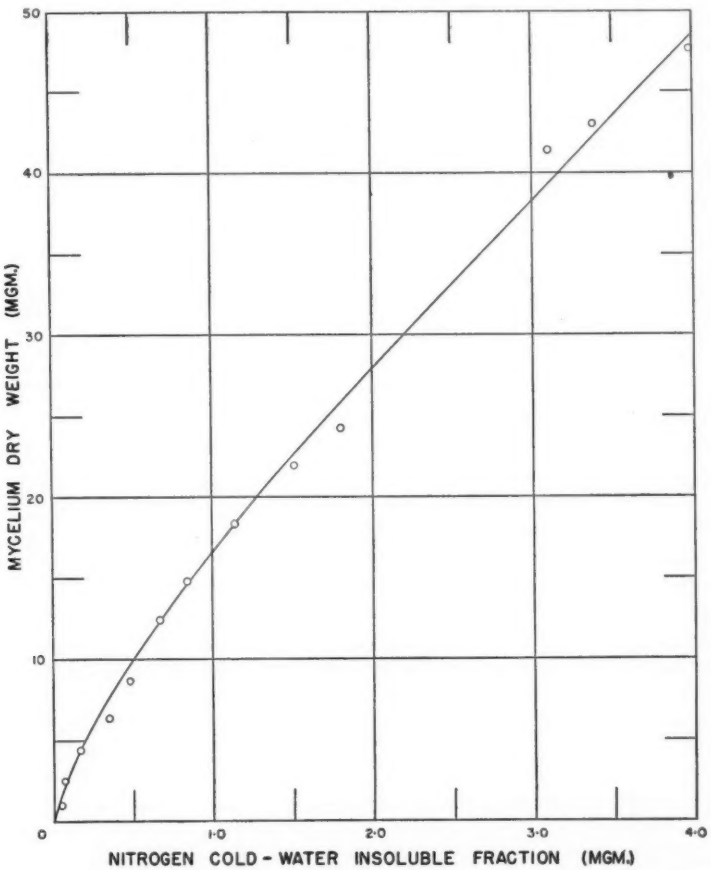

FIG. 1. Relation between cold-water-insoluble nitrogen and mycelial dry-weight of *Fomes annosus* grown on a glucose–asparagine substrate.

asparagine substrate. The residue from each jar was filtered, washed with three aliquots of cold water through a Buchner funnel, without suction, dried, and weighed. Nitrogen determinations were made of the residue by micro-Kjeldahl methods described by Clark (3), and Hiller *et al.* (8).

Experimental Results

Effect of Subculturing on the Occurrence of Variants

To investigate the occurrence of variants and to assess the degree of variation in this fungus, experiments were conducted with 12 European and 4 North American cultures by examining the amount of change in growth rate and colony appearance between successive subculturings. Four plantings employing malt agar plate cultures of the isolates were made over a period of three months. Colony appearance and growth rate were recorded for each of the isolates after a period of seven days, and the character of the colony for each subculture was compared by employing a system of descriptive terms based on definitions used by Long and Harsch (11), and Campbell (2) to describe fungal cultures. These terms, which are defined below, have been given a numerical designation so that colony appearance might be expressed by a simple numerical profile.

(1) Appressed—mycelium is prostrate on the surface of the agar.
(2) Raised —mycelium is raised in tufts or in a cottony mass.
(3) Cottony —erect mycelium spreading in all directions.
(4) Silky —long parallel threads of mycelium, more or less prostrate like combed silk.
(5) Floccose —thin, cottony pubescence, collected in very minute tufts.
(6) Fimbriate—possessing a fringed edge.
(7) Smooth —possessing a smooth regular edge.

Changes in colony appearance, growth rate, and the occurrence of variant types between four successive subculturings of 16 isolates over a three-month period are recorded in Table II. Throughout the subculturings, uniformity of media and constancy of temperature were stringently controlled; thus, alterations in growth characters for most colonies involve intrinsic variation characteristic of the parent culture, rather than the effect of these environmental factors. A wide range of variation was present both in appearance and in growth rate of the colonies, but it was observed that isolates which displayed a relatively uniform behavior with respect to colony appearance also displayed a less variable growth rate. This connection between growth rate and colony type was apparent when different plantings of the more variable isolates displaying similar growth patterns were grouped together. The spontaneous occurrence of variants which differed from the parent colony in morphological and growth characteristics can be observed in nine of the 16 isolates recorded here, marked changes frequently occurring in color, mycelial-mat characters, and growth rate among the subcultures of a particular isolate. Such changes, moreover, were generally reversible as indicated for the subcultures of isolate *A*-16 (see Table II). In this instance, the first and

TABLE II

THE EFFECT OF SUBCULTURING (FOUR PLANTINGS) IN RELATION TO THE GROWTH RATE, THE NATURE AND COLOR OF THE MYCELIAL-MAT, AND THE OCCURRENCE OF VARIANTS AMONG 16 ISOLATES OF *Fomes annosus*, RECORDED AFTER A PERIOD OF SEVEN DAYS

Culture No.	Variant	Frequency of occurrence	Mycelial-mat characteristic profile	Color	Growth* rate (mm.)	Growth† type
E-8	—	4	1 4 6 7	White	51.1 ± 1.7	*I*
E-6	—	4	1 4 6 7	White	51.3 ± 4.5	*I*
E-15	—	4	1 4 6 7	White	63.0 ± 6.2	*F*
E-10	—	4	1 4 7	Sodden gray	41.2 ± 4.1	*I*
E-12	—	4	1 4 7	White	62.7 ± 12.4	*F*
E-14	—	4	1 6 7	White	47.7 ± 5.2	*I*
E-4	—	3	1 6 7	White	52.8 ± 3.0	*I*
A-2					20.1 ± 8.2	*S*
	A-2$_1$	2	1 6 7	White	12.0 ± 2.0	*S*
	A-2$_2$	2	1 6 7	Sodden gray	28.2 ± 0.2	*S*
E-13					48.6 ± 10.0	*I*
	E-13$_1$	2	1 6 7	Gray to white	55.2 ± 4.0	*I*
	E-13$_2$	2	1 8	Sodden white	42.0 ± 10.0	*I*
E-5					58.7 ± 14.4	*I*
	E-5$_1$	1	1 4 6 7	White	82.0	*F*
	E-5$_2$	3	1 4 7	Sodden white	51.0 ± 6.1	*I*
A-3			1 2 4 6 7	White	64.8 ± 12.8	*F*
	A-3$_1$	1	1 4 7	White	44.5	*I*
	A-3$_2$	3	2 4 6 7	White	71.6 ± 2.4	*F*
E-9					67.9 ± 15.3	*F*
	E-9$_1$	2	1 6	White	53.8 ± 2.7	*I*
	E-9$_2$	2	1 6 8	White	82.0 ± 8.0	*F*
E-11					19.6 ± 12.0	*S*
	E-11$_1$	2	1 6	White	8.0 ±	*S*
	E-11$_2$	2	1 7	Sodden Yellow	31.0 ± 4.0	*I*
E-7					8.6 ± 4.0	*S*
	E-7$_1$	2	1 8	White	5.0 ± 1.0	*S*
	E-7$_2$	2	1 7	Sodden white	12.2 ± 1.2	*S*
A-1					24.2 ± 4.4	*S*
	A-1$_1$	2	2 7	White	20.0 ± 1.5	*S*
	A-2$_2$	2	1 8	Yellow	28.5 ± 1.0	*S*
A-16					44.6 ± 27.5	*I*
	A-16$_1$	2	1 6 7	Tan	19.2 ± 1.2	*S*
	A-16$_2$	2	2 3 8	Grey	70.0 ± 15.0	*F*

* *Mean growth rate of four subcultures.*
† *S–slow (colony 1–30 mm.), I–intermediate (colony 30–60 mm.), F–fast (colony 60–90 mm.)*

third, and the second and fourth subcultures of the series agreed closely in colony characteristics and growth rate, being marked by two reversions to former types during the four subculturings. On the basis of colony characteristics alone it was thus possible to distinguish two types of colony for this isolate, each possessing different growth capacities. Similarly, it was possible to demonstrate the coexistence of distinct types in over half of the isolates examined. The fact that the standard deviation of the growth rate values, calculated on the basis of the four subculturings, was often reduced by from 50 to 100% after subcultures possessing similar colony characteristics were separated, further suggested a close connection between growth rate and morphology of the variant colonies arising from transfers of a particular isolate.

Because of the excessive variation in growth rate values shown by the individual isolates in the four plantings, an attempt was made to characterize each culture on the basis of whether it could be assigned to a slow-, intermediate-, or fast-growing class (see Fig. 3). This was only partly successful since a few of the isolates produced variants that fell into adjacent classes, and subcultures of isolate *A*-16 were distributed among all three of the arbitrary growth groups. Nevertheless, it was thought that growth charac-

teristics based on what might be regarded as the dominant colony characteristic, i.e. mean colony diameter, would provide a clue to a possible relationship between the growth behavior of the isolates and host or geographic origin. The data, thus arranged, are shown in Table III. No consistent relation was seen to occur between isolates originating in Norway, which accounts for half of the cultures, and the three growth rate groups; Norwegian isolates occurred in each of the arbitrary group. The two Canadian isolates appeared in the slow-growing group but shared this position with an isolate from Germany and Norway. The two U.S.A. cultures, on the other hand, were designated as fast- and intermediate-growth types respectively. Isolates originating in European countries, as a group, occurred indiscriminately among the three types, and similarly the four North American isolates were associated with the three growth-type groups. Thus, no correlation was found between rate of growth, which was determined on the basis of the four successive plantings, and origin of the cultures; cultures displaying the three growth characteristics occurred in both the North American and European groups.

Except for isolates from *Pinus sylvestris* (*E*-4, *E*-6, and *E*-14) originating in England and Norway, which displayed similar growth characteristics in all these experiments, no consistent relation was evident between growth rate and host species. Owing to the limited number of cultures originating from the same host species, it was not possible to test this relationship further, but it seems unlikely that any connection exists between the type of colony and a particular host, since Roll-Hansen (21) in experiments with this fungus found cultures displaying different growth characteristics associated with *Pinus sylvestris* in Norway.

TABLE III

ISOLATES OF *Fomes annosus* FROM VARIOUS HOSTS AND LOCALITIES LISTED IN ARBITRARY GROUPS ACCORDING TO THEIR MEAN GROWTH RATE VALUES

Culture No.	Host	Place of origin	Growth* type	Mean colony diameter of four subcultures
E-8	*Larix* sp.	England	*I*	51.1
E-14	*Pinus sylvestris*	E. Norway	*I*	47.7
E-10	*Calluna vulgaris*	W. Norway	*I*	41.2
E-6	*Pinus sylvestris*	Norway	*I*	51.3
E-13	*Vaccinium myrtillus*	E. Norway	*I*	48.6
E-4	*Pinus sylvestris*	England	*I*	52.8
A-16	*Pinus resinosa*	Conn., U.S.A.	*I*	44.6
E-5	*Vaccinium myrtillus*	E. Norway	*I*	58.7
E-9	*Calluna vulgaris*	Norway	*F*	67.9
E-12	*Betula* sp.	E. Norway	*F*	62.7
E-15	*Picea abies*	E. Norway	*F*	63.0
A-3	*Juniperus virginiana*	S.C., U.S.A.	*F*	64.8
E-11	*Sorbus aucuparia*	W. Norway	*S*	19.6
A-2	*Tsuga heterophylla*	B.C., Canada	*S*	20.1
A-1	*Pseudotsuga taxifolia*	B.C., Canada	*S*	24.2
E-7	Pine or spruce (?)	Germany	*S*	8.6

* *I–intermediate (colony 30–60 mm.), F–fast (colony 60–90 mm.), S–slow (colony 0–30 mm.)*

Effect of pH of the Medium on Radial Growth Rate

The hydrogen-ion ranges and growth optima for cultures of *A*-1, *A*-2, *A*-3, *E*-4, *E*-5, and *E*-6 were determined to ascertain whether differences in growth rate, colony form, and place of origin were correlated with differences in tolerance to hydrogen- and hydroxyl-ions. One hundred and eight Petri dishes containing buffered malt agar adjusted to six different hydrogen-ion concentrations over the pH range 3.1 to 6.9 were inoculated in triplicate series with spore suspensions of the above cultures and incubated at 23° C. Colony diameters were measured and recorded at the end of seven days, the results representing the averages of six measurements for each culture.

Fig. 2 compares the rate of growth with the change in pH for the six cultures. Colony diameters were plotted against initial pH values and the change from initial pH due to the metabolism of the fungus was noted for each pH reading. The metabolic activity, as measured by this change, was relatively uniform for each of the cultures and the final pH values tended towards similar pH values (pH 4 and pH 6) for each of the cultures. From the growth curves of cultures of *A*-1 and *E*-5, which indicate maximal growth at pH 4.6 and 5.5, and the general tendency for the six cultures to change the initial reaction of the medium in a direction toward these two optima, double pH optima for this fungus under certain conditions are indicated.

The pH range for optimal growth of the six cultures is listed in Table IV. The range indicated by the first optimum fell within the relatively narrow limits of 0.35 pH-units between pH values 4.6 and 4.95, these limits being

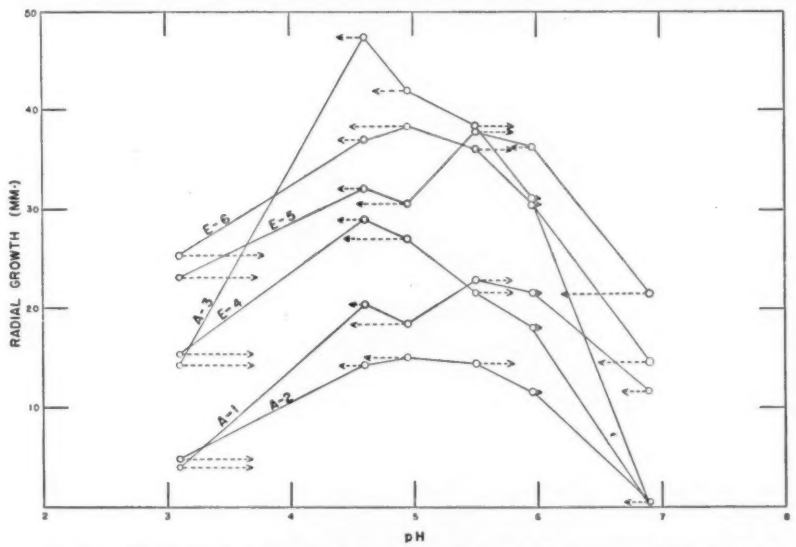

FIG. 2. Effect of pH on growth of North American and European cultures of *Fomes annosus*. Diameters of colonies on 2.5% malt agar with various initial H-ion concentrations. The broken lines indicate the pH change due to the metabolism of the fungus.

TABLE IV

HYDROGEN ION OPTIMA OF EUROPEAN AND NORTH AMERICAN ISOLATES LISTED IN ORDER OF GROWTH TYPES

Culture number	Growth-rate type	Initial pH growth* optima after seven days	
A-1	Slow	4.6	5.5†
A-2	Slow	4.95	
E-4	Intermediate	4.6	
E-6	Intermediate	4.95	
A-3	Fast	4.6	
E-5	Fast	4.6	5.5†

** Linear growth is estimated on the basis of the average colony diameter of three replicates.*
† Double growth optima.

extended, however, toward neutrality, when the second optimum was included for cultures of *A*-1 and *E*-5, but nevertheless extended over less than 1 pH unit. The most favorable hydrogen-ion concentration for growth of the three growth rate types thus fell within relatively narrow limits, and no consistent differences were observed between the growth optimum of cultures from the European and North American groups.

Because such variables as temperature, composition of the medium, type of buffer employed, and the duration of the experiment would be expected to have some effect on the metabolism of the fungus, pH optima reported elsewhere for *F. annosus* afforded little opportunity for direct comparison. Nevertheless, the close agreement between the optimal pH range of 4.6 to 4.9 demonstrated for the test cultures by these experiments and optima of pH 4.0 to 4.9 recorded for isolates of *F. annosus* examined in Europe by Weis and Nielson (25) and Jorgensen *et al.* (10), and in North America by Nord and Sciarini (13), is such as to suggest that the strains of the fungus involved differ little in their fundamental processes.

Effect of pH and Temperature on Cellulose Utilization and Mycelial Production

Quantitative analyses of cellulose utilization and mycelial production with cultures of *A*-1, *A*-2, *A*-3, *E*-4, *E*-5, and *E*-6 were conducted according to the methods outlined in an earlier section with a view to determining whether differences in behavior between North American and European isolates could be demonstrated. The experiment was designed on a factorial plan, initially employing three factors, to provide statistical treatment of strain differences at two temperatures (23°, 25° C.) and at two pH reactions (pH 4.7, 5.4). In the final analysis, owing to the loss of some of the culture data through contaminations and unsuccessful nitrogen determinations, it was not possible to include the lower temperature series in the statistical treatment of the data. The results of testing the differences between European and North American groups, the effect of pH on the group and isolates, and the differences between replicates (series 1 and 2) are presented in Table V.

PLATE I

FIG. 3. *Fomes annosus*, seven-day-old colonies on 2.5% malt agar, incubated at 23° C. Examples of slow–(*A*-16), intermediate–(*E*-5), and fast–(*E*-12) growing cultures.

TABLE V

CELLULOSE UTILIZED AT THE END OF 17 DAYS BY NORTH AMERICAN AND EUROPEAN ISOLATES OF *Fomes annosus* INCUBATED AT 25° C.

Group	Isolate	pH 4.7			pH 5.4			Total		Grand total
		Series 1	Series 2	Total	Series 1	Series 2	Total	Series 1	Series 2	
North American	*A*-1	5.3	3.7	9.0	4.1	3.3	7.4	9.4	7.0	16.4
	A-2	0.7	3.8	4.5	3.0	2.7	5.7	3.7	6.5	10.2
	A-3	13.9	8.2	22.1	9.2	7.4	16.6	23.1	15.6	38.7
	Total	19.9	15.7	35.6	16.3	13.4	29.7	36.2	29.1	65.3
European	*E*-4	5.8	6.9	12.7	5.7	8.7	14.4	11.5	15.6	27.1
	E-5	8.5	12.2	20.7	19.7	7.1	26.8	28.2	19.3	47.5
	E-6	7.9	7.7	15.6	6.0	4.7	10.7	13.9	12.4	26.3
	Total	22.2	26.8	49.0	31.4	20.5	51.9	53.6	47.3	100.9
	Grand total	42.1	42.5	84.6	47.7	33.9	81.6	89.8	77.4	166.2

NOTE: *Significance of difference between means:*

	P(*Z*)
European and North American groups	*>0.05 (<0.20 when isolate mean square is used as denominator of variance ratio)*
	<0.20
pH	*Not significant*
Groups—pH	*0.20*
Isolates	*>0.05*
	<0.20
Isolates—pH	*0.20*
Series	*0.20*

In calculating the variance ratio to obtain P, the error or individual mean square was used as the denominator, except to test group differences, where the large differences between isolates would obviously contribute to a large variation. In this case, the individual mean square was replaced by the isolate mean square as the denominator for the estimate of error.

Rather large mean squares were recorded for the isolate and group items though not one of them was significant at the 5% level of probability, the differences between isolates being greater than the differences between groups. The pH effect on both group and isolates had no statistical significance in this analysis.

TABLE VI

EFFECT OF pH AND TEMPERATURE ON THE GROWTH AND CELLULOSE DECOMPOSING POWER OF EUROPEAN AND NORTH AMERICAN ISOLATES OF *Fomes annosus* (INCUBATION—17 DAYS)

	Cellulose decomposed, mgm.					Mycelium produced, mgm.				
	23° C.		25° C.			23° C.		25° C.		
Culture Nos.	pH 4.7	pH 5.4	pH 4.7	pH 5.4	Mean	pH 4.7	pH 5.4	pH 4.7	pH 5.4	Mean
A-1	6.1	4.7	4.5	3.7	4.8	7.1	5.9	3.5	4.1	5.1
A-2	9.6	3.7	2.2	2.8	4.6	7.5	4.4	2.4	3.0	4.3
A-3	6.5*	7.3	11.0	7.1	8.0	4.2*	4.5	3.9	5.9	4.8
Subtotals	22.2	15.7	17.7	13.6		18.8	14.8	9.8	13.0	
Mean	7.4	5.2	5.9	4.5	5.8	6.2	4.9	3.2	4.3	4.7
E-4	3.3	8.8	6.3	7.2	6.4	2.9	3.9	3.2	4.1	3.5
E-5	10.2	9.9	10.3	13.4	11.0	7.1	5.6	8.0	7.9	7.1
E-6	5.9	—	6.7	7.9	5.1	4.3	—	3.2	3.9	3.8
Subtotals	19.4	18.7	23.3	28.5		14.3	9.5	14.4	15.9	
Mean	6.4	9.3	7.7	9.5	7.5	4.7	4.7	4.8	5.3	4.8
Grand total	41.6	34.4	41.0	42.1		33.1	24.3	24.2	28.9	
Mean	6.9	6.9	6.8	7.0	6.6	5.5	4.8	4.0	4.8	4.7

NOTE: *Results expressed in mgm./30 ml.*
* *Based on one flask only.*

The data were also examined for the separate effects of pH and temperature on cellulolytic and growth activity. With the exceptions noted, Table VI records the means (not treated statistically) of two replicates of each isolate, together with the average values obtained for each of these physiological processes. The average mycelial production and cellulose utilization for the three North American cultures was greater at 23° C. than at 25° C., but the reverse trend held for the three European cultures. The absence of any appreciable over-all influence of pH reaction on cellulose decomposition or mycelial production in the experiments possibly results from the choice of pH variables, both of which fall within the range for optimum growth of the test cultures. It is evident from the table that the production of mycelium (dry-weight basis) was approximately uniform among the cultures on this substrate, e.g. cultures of *A*-1 and *A*-2 which were designated "slow-growing" on malt agar approached the "fast-growing" cultures (*A*-3, *E*-5) in the dry weight of mycelium produced.

While no attempt has been made to investigate the nature of the occurrence of variants, the evidence obtained from these studies does not seem to support any suggestion that genetical changes in the cultures may contribute to this type of variation. It is possible that minor changes in colony form and color may arise simply from the effect of uncontrolled variables involving age, history, or environment of the cultures that have not been adequately investigated. Whatever may be the responsible factor, the lack of any recognizable differences in the characters studied between isolates of different geographic or host origin, suggests that such variation in this fungus is of subordinate importance and possibly would not represent any real difference in the behavior of the particular strains in nature.

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POLLEN PROFILES FROM THE QUEEN CHARLOTTE ISLANDS, BRITISH COLUMBIA[1]

By Calvin J. Heusser

Abstract

Five peat sections were excavated from muskegs on Langara, Graham, and Moresby Islands of the Queen Charlottes with the primary purpose of reconstructing the postglacial plant succession and associated climatic and physiographic alterations. A secondary purpose was to support or disprove the geological and zoological data favoring the existence of refugia in which biota survived from preglacial or interglacial time. The oldest pollen record tends to support this contention. The record is older than any derived from sections heretofore studied on the northwest coast. Twenty-three plant entities are represented in the bottom sediments below the lodgepole pine maximum which in these other sections marks the oldest peat. In addition, 27% of the coniferous pollen at the base of the section is constituted of climax forest trees, thus implying the presence of long-established forest when pollen sedimentation began. The number and kinds of pollen in the basal peat favor the interpretation that vegetation persisted in refugia through at least the last glaciation. The pollen profiles further corroborate earlier findings for changes in land–sea level relations and for the following postglacial climatic sequence: early cool-moist, warmer and drier (thermal maximum), and late cooler and wetter.

Introduction

The Queen Charlotte Islands off the northern coast of British Columbia represent an outlying segment in the region occupied by the Pacific Coast Forest Formation. The archipelago is situated equidistantly from the western and southern extremities of the forest range which extends from Cook Inlet and Kodiak Island in Alaska to California. Moreover, the group is further removed from the mainland and adjacent islands by a wider stretch of open water, while at the same time projecting further oceanward than any other major archipelago in the forest range. This isolated location of the Queen Charlottes poses interesting problems regarding postglacial biotic migration and the development of the forest and its associated flora and fauna.

Certain geological and zoological findings on the islands support the belief that glaciation of the group was not total. This evidence is drawn from areas where glacial features are absent and from distinctive animal populations, both suggesting the existence of refugia during the last glacial substage at least. Such areas in which plants and animals might persist during times of glaciation would serve as centers for postglacial dispersal; a greater antiquity of the life on the islands compared with that of the opposite mainland and contiguous islands, which show signs of intense glaciation, is implied.

Application of pollen studies to the deepest deposits of peat accumulation that could be located was made with two objectives: to extend the investigation of the postglacial plant succession with its accompanying climatic and physiographic alterations from the district of the Coast Forest Formation in

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Alaska (22, 23, 24, 26) southeastward toward the region of Vancouver Island and the Puget Lowland of Washington reported on by Hansen (15, 16, 17, 18, 19, 20, 21); and to disprove or contribute further support to the opinion favoring the occurrence of glacial refugia.

A liberal grant in 1954 from the Penrose Fund of the American Philosophical Society financed the undertaking of this work; in addition, the Juneau Ice Field Research Project which is directed by the American Geographical Society through contract with the U.S. Office of Naval Research enabled a portion of the travel connected with the investigation. Certain equipment was provided by the University of Minnesota, through the courtesy of Dr. Donald B. Lawrence, and by the American Geographical Society, through the kindness of William O. Field. To the above persons and organizations, and to the following, appreciation for aid is extended: Stephen L. Den Hartog of Wellesley Hills, Mass., who acted as assistant; Harold Ombrach of Port Clements, Queen Charlotte Islands; Philip and Jean Stace-Smith of Langara Island and Creston, B.C.; Dr. Ian McTaggart Cowan and Dr. William H. Mathews of the University of British Columbia; Dr. Hugh S. Bostock and Dr. John E. Armstrong of the Canadian Geological Survey; and Lawrence Boulton of the British Columbia Forest Service.

Location and General Description

The Queen Charlotte Islands, as a distinctive group, are located south of the Alaskan Alexander Archipelago (Fig. 1). Hecate Strait is to the east, 35 miles wide in the north and 80 miles in the south; Dixon Entrance to the north is between 30 and 40 miles across. Queen Charlotte Sound is southeast as a 140-mile stretch separating Vancouver Island.

Four islands, from north to south, Langara (North), Graham, Moresby, and Kunghit (formerly called Prevost), form an axis over 180 miles long; Louise, Lyell, and Burnaby are additional large islands on the Hecate Strait side of Moresby Island. The archipelago is essentially triangular with the base along the north shore of Graham Island, 50 miles broad, and the whole gradually attenuating southeastward to the vertex formed by Kunghit Island, about 10 miles in width.

Graham and Moresby Islands are the largest of the group. The latter comparatively narrow, except in the north, and greater in length, is deeply incised by fiords and to such an extent that in many places only several miles of land lie between the ocean and the strait. Graham Island is about as long as it is wide on the north coast (50 miles); the southern part is 25 miles in width and like much of Moresby Island is dissected by fiords. Most of the islands of the archipelago are separated by narrow channels about a mile wide, and if it were not for these, the principal members would be joined as a single unit.

The Queen Charlotte Ranges, the outstanding topographic feature, compose a unifying system. Their highest peaks according to Bostock (3) are at 3945 ft. in elevation on southern Graham Island and at 3810 ft. on northern

FIG. 1. Sketch map of the Queen Charlotte Islands and adjacent areas showing the locations of muskegs where peat sections were taken for this study.

Moresby Island, although a more recent map (10) indicates Mt. Needham (4000 ft.) on southern Graham, Mt. De La Touche (3685 ft.) on west central Moresby, and Mt. Kermode (3750 ft.) on Louise to be highest. Other parts of the ranges are at about 3000 ft., except toward the north and south extremities where summits fall to 2000 ft. and below. The ranges are generally steep and rugged, in many places forming sharp peaks and serrate ridges, although some have rounded summits, particularly those of northern Graham

Island. No glaciers are known to occur in this region; earlier in the Pleistocene, however, the mountain chain was covered at least in part by a glacier complex. These ranges Bostock (3) has named as a physiographic subdivision of the insular mountains of the western system of the Canadian Cordillera. They accordingly are part of the outer mountain belt with counterparts forming Vancouver Island, the western Alexander Archipelago, and the St. Elias Mountains.

Moresby Island is made up almost entirely by the Queen Charlotte Ranges while on Graham Island less than half of the area is so constituted. MacKenzie (30) has outlined three additional topographic provinces: northern lowland, northeastern lowland, and central plateau; Bostock (3) would include the central plateau in the ranges province. The plateau is generally the area north of Queen Charlotte City extending for a third the length of the island and situated as the middle lower third with the ranges to the west and the northeastern lowland to the east. Further, it is 1000 to 1500 ft. in elevation and characterized by low, flat-topped hills and ridges with small lakes and muskegs. The northeastern lowland is east of the Queen Charlotte Ranges, east of Naden Harbor (the western embayment of the two on the north coast) and northeast of the central plateau; it forms the upper two-thirds and half the width of the island, its boundary passing about halfway through Masset Inlet (the eastern embayment). This low terrain is poorly drained with numerous lakes and muskegs and with elevations seldom exceeding 500 ft. The northern lowland is essentially west of Naden Harbor. At elevations ranging between 500 and 1000 ft., its surface is irregular with extensive muskeg barrens. An interesting description of this province is contained in Sheldon (39).

Climate

Owing to the Queen Charlotte Ranges making up most of Moresby Island and the western upland of Graham Island, local climatic differences are to be expected particularly between the ocean border and the Hecate Strait shore. This mountain network creates only local variations, however, and the seasonal atmospheric pressure changes over the Pacific, the Aleutian low dominating from autumn to late spring and the North Pacific high during the summer, control the over-all climate.

Heavier precipitation occurs during the period the Aleutian low is dominant. Records from Cape St. James (southernmost Kunghit Island), Dead Tree Point (15 miles south of Tlell on Graham Island), Langara Island, Masset, and Sandspit, show October through December with relatively heavy precipitation while during May to September the least amounts are received (35). Climate on the ocean coast is characterized by mist, heavier precipitation, higher mean January temperature, and lower mean June temperature with respect to the eastern areas. This is illustrated by records from Langara Island on the west, Masset at mid-island, and Sandspit on the east coast. At Langara, average annual precipitation is 67 in., mean January temperatures

38° F., and mean June temperature 51° F.; for Masset these averages are respectively 55 in., 36°, and 53°; and for Sandspit 49 in., 30°, and 52°. Temperature usually does not fall below freezing near sea level; consequently, precipitation is largely rain, and snow that falls usually does not remain. Snowfall is heavy in the higher Queen Charlotte Ranges with snowbanks persisting through the summer.

Vegetation

The cool and moist climate which enables a long growing season is conspicuous in the luxuriant growth of the vegetation. Under the maritime environment trees grow to large sizes, particularly on well-drained surfaces, with trunks and branches covered with sheaths or patches of thick mosses. Undergrowth is dense near sea level but diminishes at higher elevations. The forest floor is often pocketed and raised into mounds where trees have been toppled during past storms, and rotten trunks and branches of earlier generations cover the ground along with a heavy accumulation of partially decomposed organic material. This layer of living mosses and decaying plants may be as much as two to three feet thick. Under such circumstances, the trees are of relatively poor growth, and the vegetation may be classified as transitional to muskeg. Such transitional stands appear to be the result of late-postglacial climatic change.

The western hemlock (*Tsuga heterophylla*[2]) – Sitka spruce (*Picea sitchensis*) – western red cedar (*Thuya plicata*) association of the Coast Forest Formation is predominant on the islands. This climax grouping is manifest in the vicinity of sea level and up to about 1000 ft. Between 1000 and 2000 ft. elevation, according to Osgood (33), the composition changes gradually while above 2000 ft., the change is rapid. Western red cedar is no longer encountered and few individuals of spruce and western hemlock are found. An alpine hemlock (*Tsuga mertensiana*) – Alaska yellow cedar (*Chamaecyparis nootkatensis*) faciation dominates the upper zone of the forest. Timber terminates at variable elevations at different places. MacKenzie (30) states that the upper tree limit is usually over 1300 ft. elevation, while Osgood (33) claims that the forest reaches to about 2500 ft. with a few western hemlocks, spruces, and lodgepole pines (*Pinus contorta*) persisting on the higher ridges in the alpine zone. Sudworth (41) notes that mountain hemlock and Alaska yellow cedar extend to 5000 ft., although this reference is obviously in error as the highest peak on the Queen Charlottes is only at 4000 ft. An estimate of the highest elevation to which trees are to be found growing is thus given between 3000 and 3500 ft.

Western hemlock is the most abundant tree in the low level forest. It is not the largest and specimens probably do not attain any greater size than 3 to 4 ft. d.b.h. (diameter breast high) and 130 to 150 ft. in height. Sitka

[2] *The most recent catalogue dealing with the flora of these islands is that published by the Provincial Museum of Natural History (36). Because this treatment is antiquated and incomplete, the nomenclature of Hultén (27) is followed, since the coverage of his flora may be extended from Alaska to include this area. Where Hultén does not apply, the authority follows the binomial.*

spruce, of secondary abundance, is the largest tree and frequently 6 to 8 ft. in diameter with heights from 150 to 200 ft. Spruce on the Queen Charlottes is of the finest quality growing in the British Columbia coastal district, and western hemlock is also superior to that found in the southern provice (44). Western red cedar, of least abundance compared to the other two, grows to large sizes but is often not of sound quality. Mountain hemlock and Alaska yellow cedar usually do not reach any noteworthy size. No Douglas fir (*Pseudotsuga taxifolia* (Lamb.) Britt.) nor true fir (*Abies*) is known on the Queen Charlottes. Sudworth (41) records the presence of lovely fir (*Abies amabilis*) but this report has never been confirmed.

The Pacific yew (*Taxus brevifolia*) is a minor associate, usually not growing far from the shore. Underbrush near tidewater is often largely salal (*Gaultheria shallon*), and thickets of salmon berry (*Rubus spectabilis*) are common. Additional species conspicuous in the inland forest shrub layer are rusty menziesia (*Menziesia ferruginea*), blueberries (*Vaccinium ovalifolium*, *V. parvifolium*), blue currant (*Ribes bracteosum*), and devil's club (*Oplopanax horridus*). Several deciduous trees frequently occur on the coast and along the inland water courses: alder (*Alnus oregona*), willow (*Salix scouleriana*), and Oregon crab apple (*Malus fusca*).

Lodgepole pine is not present in the climax forest since it is unable to compete successfully. It grows in open places on rocky bluffs near the shore, in the alpine zone, or on muskegs. In these situations its growth is poor. Where drainage is less favorable to the climax conifers, the luxuriance of the forest is reduced, but in that lodgepole is favored at the same time, the tree exhibits here its best growth. Drainage of the muskeg proper is poor by comparison, and lodgepole and other trees grow low and misshapen.

Muskegs abound wherever the drainage is sluggish. Three types, flat, domed or raised, and slope muskegs, as deposits of incompletely decomposed sedge, moss, and ligneous (forest) plant parts are displayed on the islands. Mosses and sedges, in particular, and ericaceous plants now growing at the surface contribute most to the peat accumulation. Trees are sparse on the muskegs, although in the past they were more widespread at certain localities, as indicated by their remains in the deposits. The existing cool and moist marine climate greatly favors the formation of peat through a long growing season and repression of activity by decay microorganisms.

A strand vegetation thrives along stretches of the peripheries of the islands. Where the forest edge abruptly coincides with a rocky coast at the upper tidal limit, however, strand plants often find no suitable habitat. In the vicinity of Sandspit on Moresby Island and along the eastern and northeastern coasts stretching to Rose Point on Graham Island, this vegetation flourishes, consisting of such plants as the following: beach ryegrass (*Elymus arenarius* spp. *mollis*), saltweed (*Atriplex gmelini*), glasswort (*Salicornia herbacea*), sandwort (*Honckenya peploides* spp. *major*), beach pea (*Lathyrus maritimus*), and seaside plantain (*Plantago maritima* spp. *juncoides*). According to MacKenzie (30), Spit Point near Sandspit is formed by the northward sweep of the tides

coupled with the strong southeast gales that blow from Hecate Strait; Rose Point and its continuation, Rose Spit, result from the meeting of currents moving eastward through Dixon Entrance and northward up Hecate Strait. These localities appear to be continually building northward, thus increasing the area occupied by strand vegetation.

Alpine vegetation, the last type to be mentioned in this section, was previously alluded to under the discussion concerning the maximum elevation at which trees are established. This plant cover is generally above 2500 ft. but naturally varies with the upper limit of the conifer forest and occurs only on the higher summits. Osgood (33) writes that four principal mountain areas are occupied by alpine vegetation: southwestern Graham Island, Louise Island, and Moresby Island, generally opposite Louise and Lyell Islands. Representative plants of this zone are the heaths: *Cassiope stelleriana*, *C. mertensiana*, *Phyllodoce glanduliflora*, *Vaccinium caespitosum*, *V. uliginosum*, and *Loiseleuria procumbens*.

Muskeg Localities and Peat Sections

Langara Island muskeg (No. 1) is near 200 ft. elevation, approximately a mile from Henslung Bay on the southern part of the island. It is reached by a trail that connects the bay and the lighthouse at Langara Point on the north coast. An undulating topography with east–west ridges transecting the trail characterizes this southern portion. In a trough between two such ridges the muskeg has developed in an elongate basin. The surface is slightly raised and slopes gently toward either end.

The deepest section of the five muskegs reported in this paper, measuring 7 meters in length, was excavated from this deposit (Fig. 2). Inorganic sediments consisting of gray clay changing below to sandy clay form the bottom half meter of the section. Above this is a meter and a half of limnic peat which grades upward into sedge peat. Diatoms, colonies of *Pediastrum*, and other algae such as *Cosmarium* and *Staurastrum* along with sponge spicules and nymphaceous trichomes, compose much of the limnic sediments. The pollen record begins at 6.6 meters, and below this diatom frustules were the only plant materials found in the clay. No volcanic ash horizons were intercepted at this locality or the others of this study.

Two meters of ligneous peat occur in the middle third of the section. This deposit was difficult to penetrate with the sampler, in particular the 4-meter level which consists of relatively sound arboreal remains. Mixed moss and sedge peat constitutes the uppermost 2½ meters of the section; between 1.4 and 2.7 meters, bodies resembling ascomata of *Microthyrium*, as described for peats of the British Isles by Godwin and Andrew (14), were noted. These occur in the transition zone where the peat type changes; they have also been observed in muskegs in coastal Alaska (24, 26). Polypodiaceous sporangia were found throughout most of the section, particularly in the samples from 5.8 to 6.1 meters and 3.9 to 4.5 meters. Polypod spores correspondingly

increase at these levels as shown in the accompanying profile. Decomposition resulting from prolonged oxidation was not noted for any of the samples from the section.

Crowberry (*Empetrum nigrum*) is conspicuous on the surface of the muskeg along with sphagnum mosses. Several heaths, other than crowberry, were observed growing with the above plants; Labrador tea (*Ledum palustre* ssp. *groenlandicum*), swamp laurel (*Kalmia polifolia*), mountain cranberry (*Vaccinium vitis-idaea*), and swamp cranberry (*Oxycoccus microcarpus*); salal forms a dense undergrowth along the muskeg margin and on the upland as well. Other low plants noted were juniper (*Juniperus communis* var. *montana*), sedge (*Scirpus caespitosus* var. *austriacus*), cloudberry (*Rubus chamaemorus*), bunchberry (*Cornus canadensis*), sundew (*Drosera rotundifolia*), and star flower (*Trientalis europaea* ssp. *arctica*). Lodgepole pine, western red cedar, and western hemlock are growing on the peat, although all are with poor form and no more than 10 ft. in height.

On southern Graham Island, a raised muskeg (No. 2) was sampled near Queen Charlotte City. This site is on the ridge summit at about 1100 ft. elevation directly behind the village. It is circular in outline, approximately 150 ft. across, and is domed and sloping radially toward the surrounding forest. A few small pools are present on the surface.

The section is 6 meters in length (Fig. 2). Moss peat has formed more than the upper half, and the domed appearance of the muskeg is probably a consequence of this accumulation. Some sedge peat is intermixed and also constitutes from one half to a meter of the basal organic sediments. Somewhat more than a meter of ligneous peat is found between this sedge material and the upper bryophytic remains. A light brown clay grading downward into gray clay forms the bottom one half meter. *Microthyrium*-like structures are recorded for the 5.0-, 4.6-, 1.3-, and 1.2-meter levels. No evidence of charring or oxidation is apparent.

The relatively high elevation of this muskeg is indicated by the mountain hemlock and Alaska yellow cedar present on the surface. These are the principal trees along with lodgepole pine. Hemlock and cedar are generally growing erect whereas pine is low and bushy. Sedge (*Scirpus caespitosus* var. *austriacus*) in thick clumps covers the muskeg in places while sphagnum mosses and heaths, crowberry, Labrador tea, swamp laurel, swamp cranberry, and bog blueberry (*Vaccinium uliginosum*) are more evenly distributed. Cloudberry, bunchberry, western tofieldia (*Tofieldia occidentalis*), sundew, star flower, and great burnet are also worthy of mention.

Tow Hill muskeg (No. 3), on the north coast of Graham Island, is reached by road which stretches about 15 miles east of Masset. It rests close to sea level and is just west of Tow Hill, a basaltic erosional remnant rising conspicuously 425 ft. from the low-lying plain. The deposit is extensive, occupying several square miles. A 2.5 meter section was removed, the base of which consists of clay sand (Fig. 2). Ligneous peat measures almost one-half meter of the section above this while the uppermost 2 meters are mixed moss and

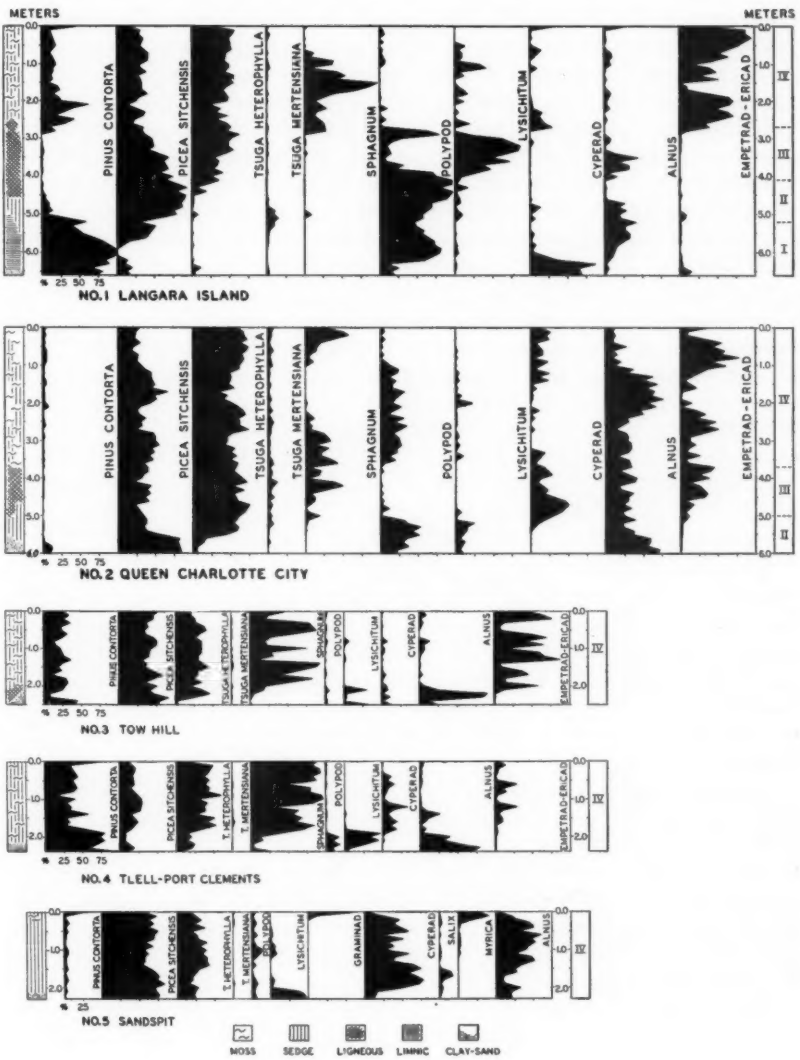

FIG. 2. Peat sections, pollen profiles, and environmental periods for Langara Island, Queen Charlotte City, Tow Hill, Tlell – Port Clements, and Sandspit muskegs. The procedure followed in constructing the profiles is explained in the text under Methods.

sedge. Black fragments of charred peat resulting from past local burning occur at 1.9, 1.4, 1.3, and 0.2 meters. Fungal bodies resembling those of *Microthyrium* are distributed in the sediments accumulated on the ligneous peat.

Depauperate lodgepole pine and western hemlock comprise almost all the arboreal vegetation on the muskeg. These plants are not more than a few feet in height. Sitka spruce and Alaska yellow cedar are occasional and exhibit the same impoverished condition. The muskeg consequently has the appearance of an extensive barren. The sphagnum moss cover is supplemented largely by heaths, including crowberry, Labrador tea, bog blueberry, bog rosemary (*Andromeda polifolia*), and salal. Low juniper, cloudberry, great burnet, and bunchberry contribute some additional cover.

The road that connects Queen Charlotte City and Port Clements crosses the northeastern lowland of Graham Island after leaving Tlell. Three miles northwest of Tlell a section (No. 4) was taken to 2½ meters (Fig. 2). The deposit consists of sedge and moss remains with the former prevalent toward the bottom of the section and the latter at and near the surface. The underlying material is fine sand. All levels above the basal half meter contain charred particles except the surface. Burned tree trunks are standing on the muskeg as well as throughout the local upland and suggest that burning occurred as recently as several decades ago. Evidently fires have occurred in this region from some time in the past up to the present century.

Sphagnum mosses dominate the surface. Crowberry, Labrador tea, cloudberry, and sedges (*Scirpus caespitosus* var. *austriacus* and *Carex pauciflora*) are noteworthy associates. Juniper, swamp gentian (*Gentiana douglasiana*), bunchberry, bog rosemary, swamp cranberry, western tofieldia, star flower, great burnet, and sundew are present although in fewer numbers. Lodgepole pine is the sole tree species on the peat; on the upland ridges it commingles with western red cedar where it undoubtedly persists owing to repeated burning.

A section from a peat deposit bordering a private logging road about four miles south of Sandspit (No. 5) was the only one taken from Moresby Island. This locality, as a narrow curved basin with a central stretch of open water, is formed directly behind the beach. The section removed was 2.3 meters and compares in length with the two preceding sections (Fig. 2). Sedge peat forms almost the entire accumulation of sediments; a small quantity of moss peat has accrued in the upper 3 decimeters while clay at 2.1 meters changes to coarse sand at the base.

Sweet gale (*Myrica gale*) is most abundant in the plant cover growing on the peat. Sedges (*Carex* spp. and *Eriophorum* sp.), sundew, marsh cinquefoil (*Potentilla palustris*), and several low Sitka spruces are additional. Pond lily (*Nuphar polysepalum*) is prevalent on the water.

Methods

The pollen profiles were constructed from counts of the microfossils at decimeter levels in each muskeg section. Between 100 and 150 grains form

the basis for calculating the percentage representation of the conifers in the diagrams; 100 microfossils of non-coniferous plants were counted, the percentage of each group as shown being figured separately from that of the conifers. Pollen and spores were identified under a binocular compound microscope using magnifications of 100× and 440×. Samples of the peat, secured in the field with the aid of a Hiller borer, were prepared for examination in the laboratory. The potassium hydroxide treatment of boiling the peat for a few minutes in a dilute solution to which methyl violet stain had been added was followed. A few drops of centrifugate, obtained by subsequently straining and centrifuging the boiled material, were then mixed in hot glycerine gelatin mounting medium under a cover slip. Samples so prepared were from the surface as well as successive decimeter levels in the muskegs and included the bottom clays and sands.

Pollen Profiles

Langara Island section (No. 1, Fig. 2), as shown by the microfossil content, reveals the oldest record of vegetation of the five localities reported in this study. Furthermore, this record projects further into the past than any sections inspected heretofore on the British Columbia and southeastern Alaskan coasts. Pollen sedimentation below the 6 meter level or lodgepole pine maximum in Period I[3] is the significant portion absent from earlier sections. Two muskegs (4.3 and 3.8 meters deep), for example, near Ketchikan, 100 miles northeast, originated during this maximum (22); an unpublished 5.6-meter section from Prince Rupert, 110 miles east, does not penetrate the lower pine zone but begins some distance above. Lodgepole is predominant at the base of the Langara Island section, although pollen of the climax conifers, western and mountain hemlock and Sitka spruce, represents 27% of the arboreal flora. The pine maximum is about one half meter higher where such an abundance relative to the other conifers is reached that dominance of pine becomes total. The profiles from the lowest half meter of peat therefore reflect the rising importance of pine along with polypods (polypodiaceous ferns) while spruce, the hemlocks, cyperads (sedges), and the empetrad–ericad group (heaths) decline. Additional pollen of *Sparganium*, graminads (grasses), *Lysichitum*, *Salix*, *Alnus*, chenopods (chenopodiaceous plants), Caryophyllaceae, *Nuphar*, *Polemonium*, *Plantago*, and *Artemisia* with other Compositae was identified in the basal peat. This lower portion of Period I may be considered a late-glacial subdivision; not until earlier deposits can be located, however, will a breakdown of this interval be attempted.

Period I under a cool and moist climate in general corresponds with pi n predominance. As terrain which had been occupied by local ice became available, lodgepole invaded. While this tree produces greater amounts of pollen than do spruce or hemlocks and is therefore highly overrepresented in the deposit, the predominance of lodgepole in Period I is nevertheless still

[3] *The use of periods to categorize the environmental and floristic events is followed as an aid to interpretation, and assigned boundaries are not absolute.*

apparent. The re-entrant in the spruce profile opposite the pine maximum at 6.0 meters is probably not indicative of the subordination shown if pine over-representation is taken into account. The important fact brought out in the profiles is that when this basin began accumulating peat, climax spruce and hemlocks comprised over one-quarter of the coniferous pollen. Even at the level of the pine maximum, although not observed in the course of the count, pollen of spruce and hemlocks is nevertheless present. These data intimate that climax forest was nearby before, during, and following the maximum.

Spruce in the upper portion of Period I became increasingly important along with mountain hemlock and alder and in Period II with polypods exhibits supremacy. Environmental conditions improved with the succession of lodgepole to spruce. Climate became warmer and drier as shown in part by the development of ligneous peat in the section. The land surface that had been undergoing alteration as the ice retreated became sufficiently nearer stable equilibrium to permit the development of small areas of climax forest. Lodgepole by this time had been restricted to localities where it was not in competition with the aggressive spruce.

Western hemlock during Period III is the last of the conifers to gain superiority. This tree requires an adequate supply of organic material in the soil, and such an edaphic environment favorable to hemlock establishment had been developing through the years of spruce occupation. Hemlock gradually attained climax proportions with spruce. Stands of spruce widespread during Period II were infiltrated by hemlock and codominance was reached at least. Maximum warmth and dryness (thermal maximum) are demonstrated by the extensive layer of ligneous peat interbedded in the muskeg. Trees grew on the muskeg in this episode; skunk cabbage was prominent on the local forest floor and polypods were probably noteworthy associates, for this latter representation appears to be overshadowed by *Lysichitum*. Muskegs that had begun to form in Periods I and II were consequently reduced in area. Alder displayed minor importance in Period III as in the foregoing while subsequently its profile is relatively poorly developed.

Climate deteriorated with the opening of Period IV. Relative coolness and wetness followed the thermal maximum. A moss and sedge peat deposited at the time and resting above the ligneous sediments is evidence for this change. Forest that had earlier invaded muskeg could no longer persist on such poorly drained sites; marginal areas became occupied by muskeg with the result that the area covered by the accumulation of moss–sedge peat increased. Lodgepole pine was accordingly favored during the late postglacial as shown by its rise in the profile, since it could thrive on the peat. The presence today of lodgepole in transitional stands where muskeg had invaded the forest is indicative of this situation. *Sphagnum* and heath profiles are most pronounced in this upper portion of the section in keeping with a cool and moist climate. The differentiation of a subsequent period (V), as was done in earlier Alaskan studies (22, 24) to correspond with the climatic change which brought about general glacier retreat since the mid-eighteenth century, does not seem justifiable.

An interesting feature, distinctive of Langara Island muskeg, is the limnic peat resting as the bottom meter and a half of sediments. Previous muskegs studied, including those of this study, have not displayed a lacustrine origin; instead genesis occurs, usually with sedge peat being deposited. Through a sequence of peat types this muskeg developed from a water-filled basin. Diatomaceous and other algal material occurs throughout the limnic accumulation along with pollen which recorded the early hydrarch succession, later to evolve from limnic, to sedge, to ligneous peat. Apparently a sedge mat with occasional willows grew from the periphery while pond lily and bur-reed (*Sparganium*) occupied the open water. No pollen of these hydrophytes nor algal material is to be found above 5.1 meters, and the sedge peat which is first noted at this level suggests convergence of the mat. Succession was completed when arboreal plants occupied the muskeg during the thermal maximum; subsequently reversion has taken place to sedge–moss peat.

Queen Charlotte City muskeg (No. 2, Fig. 2) is almost as deep as the Langara section although it originated much later as sedge peat accrued during the spruce maximum of Period II. Only a small proportion of lodgepole at the base of the profile suggests a remnant of the earlier pine zone. The hemlock–spruce forest supersedes in Period III and registers only minor fluctuations in composition upward to the surface. By comparison to Langara profiles Period IV lodgepole is weakly represented while mountain hemlock is slightly more abundant. These variations are probably a result of the higher elevation of the Queen Charlotte site; also in the case of lodgepole, lower precipitation and the location of the muskeg directly above the well-drained ridge front have not been conducive to any expansion of this species. Further comparison of the two muskegs shows that *Sphagnum* reaches its peak later and is also more abundant in Period III; polypods are more prominent in Period IV; *Lysichitum* does not develop as conspicuously; trends for cyperads and the heaths are generally similar, both showing a wide over-all distribution; and *Alnus* is strongly displayed with prominences in Periods II and IV, the former occurrence resulting from participation of the genus in early post-glacial succession and the latter from invasion of disturbed areas created in the nearby Queen Charlotte Range as the snowline lowered ("little ice age").

Interpretation of Tow Hill, Tlell – Port Clements, and Sandspit profiles and sections (Nos. 3, 4, and 5 respectively, Fig. 2) places these muskegs as beginning simultaneously during Period IV. If comparison of the Tow Hill and Tlell – Port Clements profiles is made at approximately equal depths with those from Langara, general similarity can be seen. In the case of Tow Hill, *Sphagnum* and *Alnus* are more important, and in that of Tlell – Port Clements this is likewise true while the heaths are poorly represented. Chenopodiaceous pollen occurs abundantly in the lowest Tow Hill peat.

Extreme difficulty was encountered in obtaining adequate pollen counts from Tow Hill material. This may be caused by the prevailing southeast winds picking up only small amounts of pollen from the vegetation on the narrow strip of land (six to eight miles across) between Hecate Strait and

Dixon Entrance; similarly, any wind from Dixon Entrance would probably carry little or no pollen. Pronounced fluctuations in the profiles from these two localities are no doubt a consequence of frequent past fires. Peat typology of the sections where it applies to that of Langara is identical.

Sandspit conifer profiles at comparable depths are likened to those from Queen Charlotte City. Diagrams were necessarily altered since microfossils of *Sphagnum* and heaths were so few that profiles could not be constructed. *Salix*, *Myrica*, and graminads replace these. Cyperads show up more prominently at Sandspit, and the abundance of grasses and *Myrica* in the upper levels indicates recent changes in the surface flora of this locality. The prevalence of spruce in proximity to the coast is illustrated by the Sandspit and Tow Hill profiles. Hemlock, on the other hand, is more common inland as at the Tlell – Port Clements locality. The scarcity of muskegs on southern Graham and northern Moresby Islands (Queen Charlotte and Sandspit, respectively) as contrasted with northern Graham (Tow Hill and Tlell – Port Clements) where they cover extensive areas is substantiated by the scantiness of lodgepole pine.

Nature of the Evidence in Support of Glacial Refugia

Geological

The geology of the Queen Charlottes has been studied largely in connection with mineral deposits. Observations of features and materials related to the Pleistocene are generally incidental in reports on the economic geology, and most work was done during the first quarter of this century. Graham Island has been the focus of attention whereas other islands have received far less consideration. Dawson (9), Ells (13), Clapp (5), and MacKenzie (28, 29, 30) have furnished the bulk of what is known about glaciation. Dawson's report and the final report of MacKenzie are the most informative of the above sources.

That multiple glaciation affected the islands is not discussed by these authors, although Dawson mentions two glaciations on the mainland, the first of which was most intense. His conclusions are corroborated by the recent work of Armstrong and Tipper (1). From this it is reasonable to assume tha these two also occurred on the Queen Charlottes, and since no differentiation of the glacial material appears to have been attempted, it is uncertain to which glaciation biotic relationships are to be drawn. This is the first important point to be made as it bears on the refugium argument in that the latter less intense glaciation (probable late Wisconsin) may not have gained the extent of the previous glaciation. Assuming that biota present prior to the first glaciation were completely obliterated but that during the interim between this and the last glaciation they became re-established, survival may have been possible beyond the limits of the latter ice. No evidence of glaciation is found at certain places, and it is further a possibility that existing biota have been derived from these areas of the preglacial land mass.

The islands apparently constituted a center for local glaciation. Ice from the Queen Charlotte Ranges flowed out through the fiords predominantly westward and eastward. Such waterways as those separating Graham, Moresby, and Kunghit Islands are thus scoured and grooved along their borders. Parts of upper Masset Inlet (eastern embayment of northern Graham Island) show signs of glaciation as do the islands west of the mouth of Masset Sound (connecting the inlet with Dixon Entrance). Erratics are found on much of the northern and eastern coasts of Graham. The northeastern portion of the island is mantled with stratified drift consisting of clays, sands, and gravels along with unstratified sand and gravel containing striated boulders up to three feet through. MacKenzie (30) believed that the stratified sediments were laid down in lakes and estuaries brought about by glacial damming while the unstratified drift was associated on the upland. He did not concur with Dawson (9) who advocated a glacial marine origin. Dawson states that the sea level relative to the height of the land was 200 to 300 ft. higher while glaciation was active. A piedmont lobe apparently afloat covered the northeastern lowland and was probably coalesced with shelf ice from the mainland. The situation here appears to be much the same as that which prevailed on Middleton Island in the Gulf of Alaska as shown by Miller (32). Glacial material on this island has been derived from floating ice and subsequently emerged above sea level.

Glaciation has differentially modified the islands, although in most places it seems to have been local or generally weak. MacKenzie (30) made observations in the vicinity of Yakoun Lake, south-central Graham Island, and reached the conclusion that many of the surfaces were little altered or unglaciated. Valleys tributary to the lake are V-shaped and moderately if at all ice eroded. The lake itself at less than 500 ft. elevation is of preglacial origin, its islets not having been overridden. In the Queen Charlotte Range, slopes and summits have been glaciated only in part, and glaciation appears not to have been widespread above 3000 ft. If tree growth reaches to from 3000 to 3500 ft. elevation at present, as stated earlier, arboreal as well as other plants could have endured in the marginal zone. Isolated specimens of Sitka spruce, for example, have been observed growing as high as 3900 ft. on nunataks bordering Taku Glacier in the Juneau Ice Field in southeastern Alaska (25). As a second important point therefore: if parts of the upland were not covered by the glacier complex, it seems reasonable to assume, in view of existing distributions, that plants could survive there and tolerate the rigor of the environment.

According to Dawson (9) little or no evidence of ice passage over the islands is to be found. Erratics noted near sea level were no doubt transported from the mainland or neighboring islands by means of ice rafting. Cordilleran glaciers flowed off the Coast Mountains and discharged into the tidal waterways as shown by the studies of Buddington and Chapin (4) in Alaska and of Dolmage (11, 12) in British Columbia. Armstrong and Tipper (1) suggest that possibly the greatest accumulation areas for glaciers were on the Coast

Mountains lying opposite Dixon Entrance and Queen Charlotte Sound. These portions should have received greater precipitation since no orographic barriers lie westward to intercept the moisture-laden winds. The entrance and sound were also a means of exit for amassing ice. Shelf ice from the mainland probably did not have an opportunity to build up on the Queen Charlottes owing to these broad means of egress into the Pacific.

Along the ocean coast even less of an opportunity was afforded by which ice could accumulate. Any ice shelf that may have developed would become afloat owing to the deep 500-ft. ocean lying almost directly off shore with depths of 8000 to 9000 ft. in 20 miles. The adjoining coastal mountains reaching several thousand feet provide a continuation to the sharp declivity of the submarine profile (34). Under such topographic conditions glaciers would have flowed directly into the ocean and would have been restricted from thickening and spreading laterally. This reasoning provides the final point to be presented in this section: on the headlands between the fiords through which ice flowed, biota could have persisted on ice-free surfaces. This reasoning was earlier developed in the phytogeographical studies of Dahl (7, 8) for analogous situations on the Norwegian coast.

Zoological

A distinctive fauna abounds on the archipelago. Osgood (33) found that land mammals that he studied could be differentiated from species of the same genera on the mainland. His findings have been partially supplemented by McCabe and Cowan (31) while at the same time the nomenclature he followed has been partially revised by these authors. Well-defined mammalian species are: caribou (*Rangifer dawsoni*), marten (*Martes nesophila*), and weasel (*Mustela haidorum*); races include: black bear (*Ursus americanus carlottae*), white-footed mouse (*Peromyscus sitkensis prevostensis*), and shrew (*Sorex obscurus prevostensis*). In the avian fauna distinct races of Steller jay, pine grosbeak, hairy woodpecker, and saw-whet owl have been separated. McCabe and Cowan state that insular isolation for a longer interval than that of postglacial time has been necessary to develop this fauna. They conclude that these animals have evolved on the islands from a preglacial or interglacial stock.

One of the most remarkable and controversial finds was that of a new species of caribou as described by Seton-Thompson (38). Osgood (33), who early checked the report, could find no conclusive evidence for the presence of this animal. He attributed the head and bones, from whence the description came, to a hoax and the eye-witness reports to native (Haida) myths. In 1906 Sheldon (39), however, pursued the matter further. Although he was unsuccessful in finding caribou, he nevertheless did locate tracks and droppings of the animal. After his visit, three caribou were discovered and shot in 1908. Skins and skulls were sent to the Provincial Museum in Victoria and mounted. Since then no reports have been furnished, and it appears that the species has become extinct or individuals are now only few in number. Caribou

are high latitude, interior continental animals and do not range today in the southeastern Alaska and British Columbia coastal region. The Queen Charlotte caribou represents an extraordinary disjunct population, the speciation of which has been recently discussed by Rand (37).

If it is argued that animals on the islands have instead become established in postglacial time and distinctive characters have evolved through this relatively short period of isolation, difficulty is experienced in explaining how migration took place from the mainland. Hecate Strait is a formidable barrier to migration. As Osgood (33) has pointed out the strait is too broad to be crossed by swimming animals, and it is highly improbable that a crossing on driftwood could have been negotiated, even if the animal could survive, owing to the strong tides moving the length of the strait rather than across it. He points out further that the presence of marten, a terrestrial animal, and the absence of semiaquatic mink and deer, which are given to swimming considerable distances, indicate that migration was during an earlier time of land connection. The case of the aforementioned *Peromyscus* and *Sorex* as occurring on Kunghit Island but nowhere else on the Queen Charlottes, even though Kunghit is separated by less than a mile from Moresby Island, is pertinent. Certainly if these animals have ostensibly not crossed this channel they could not be expected to have crossed 35 to 80 miles of strait.

Following Osgood's thesis which seems the only one tenable that the islands were populated with animals at a time of complete mainland connection, an explanation is offered in support. Assuming that approximately the same water barrier existed in preglacial or interglacial time, at the beginning of the glacial intervals with sea levels lower, as ice was building up over the continents, Hecate Strait might have been crossable. Hecate Strait is relatively shallow by inspection of U.S. Navy Hydrographic Office Chart 1583 (43). For 25 miles off the northeast coast of Graham Island a route can be followed to within three or four miles of the islands of the Tree Knob Group and no depths greater than 72 ft. are encountered. This last stretch has depths to about 350 ft. Beyond the Tree Knob Group, the mainland in the vicinity of the mouth of the Skeena River, east of Prince Rupert, can be reached by crossing via Stephens, Porcher, and several smaller islands, and the widest channel is only two miles from shore to shore. It is an accepted fact that sea levels were several hundred feet lower during glacial times, the amount of lowering varying in accordance with the magnitude of the glaciation. Waterways separating the Queen Charlottes from the mainland during periods of lower sea levels must have been narrow or even complete connection may have been reached. Existing waterways could easily have been crossed by animals accustomed to swimming or by means of driftwood for those strictly terrestrial types. McCabe and Cowan (31) resort to slide rafts as an important vehicle used in bringing about the present distribution of *Peromyscus*. Such rafts result from dynamic slippage of earth, humus, roots, branches, trunks, and various other growth. Their occurrence is well documented along the inlets and among the islands of the British Columbia coast. Animals have been

observed among these rafts, and it seems completely plausible that this slide debris could have acted as a means for transporting animals at times of low sea level as well as at present.

This information points to the correctness of Osgood's reasoning and the preglacial origin of the fauna as expressed by McCabe and Cowan. Animals that had become isolated on the Queen Charlottes as glaciers advanced across Hecate Strait must have been able to pass at least the last glacial period successfully and evolve into the present well characterized forms. As sea level eustatically rose in postglacial time, communication with the mainland was blocked and has since remained so. Certain animals such as deer (recently introduced by man), mink, red squirrel, and red-backed and meadow mice, which are absent from the islands, may have abounded in the preglacial or interglacial period but did not survive glaciation.

Discussion

Botanical evidence contained in the peat of the Langara Island section tends to supplement rather than disprove the preceding geological and zoological data favoring glacial refugia. The earliest pollen record begins with at least 23 entities, belonging to the pteridophytes and seed plants, being represented; considering that pollen in the lowest half meter, four additional genera can be added.[4] Such is an unexpected number of plants to find present at the very inception of pollen sedimentation, if it is assumed that total glaciation occurred on the Queen Charlottes. Instead of a gradual increase of species, as would be expected with migration and succession on a deglaciated surface, the striking assemblage above is suddenly exhibited.

Because the circumstances necessary for the dispersal and migration of a plant in this case are so complex, the successful establishment of a diaspore would be highly fortuitous. The diaspore would have had to come from beyond the glacial border in Washington, 600 miles distant. It could not have come from the opposite mainland since, as it will be recalled, Langara Island profiles are older than those from Ketchikan and Prince Rupert and consequently imply that plants arrived on the mainland much later owing to a lingering glacier cover. This same contention holds if it is reasoned that the base of the Langara section may have been truncated and does not begin with the earliest plant record for the locality, and further, that the numerous kinds of pollen present when peat began to form are from plants that represent the product of a long period of migration.

Spruce and hemlock pollen amounting to as much as 27% of the coniferous flora in the earliest peat at Langara furnishes evidence that climax forest thrived during the period lodgepole pine was invading the deglaciated land. This information makes credible the existence of long-established forest that

[4] *Constituting the record in the earliest peat: 4 spp. polypods, Pinus, Tsuga heterophylla and T. mertensiana, Picea, Sparganium, graminads, cyperads, Lysichitum, 2 ssp. Salix, Alnus, chenopods, Caryophyllaceae, Nuphar, empetrads–ericads, Polemonium, Plantago, and Artemisia with other Compositae; additional from the lowest half meter: Lycopodium, Rumex, Epilobium, and Cornus.*

persisted from preglacial or interglacial time as part of the vegetation of the nunatak complex. It further offers an explanation for the early arrival of a wide variety of pollen: plants from which this pollen was derived were associated with the above forest and other type vegetation present in the refugia.

The past relationship between sea level and northeastern Graham Island has according to Dawson (9) been altered so that the present land surface stands an estimated 200 to 300 ft. higher than formerly. Late-glacial land–sea level variation is known to have been widespread along Pacific coastal Alaska (42) and British Columbia (2, 40). This change is helpful in attempting to solve certain attendant problems. Transgressed marine waters were as effective as glaciers in reducing the area of inhabitable land mass. The origin of Langara peat at about 200 ft. elevation is envisaged as following the withdrawal of the sea. The island has a maximum elevation of 523 ft. and shows no indication of having been glaciated. The portion unaffected by land–sea level variations, as a refugium, is presumed to have provided the source of much, if not all, of the earliest pollen at the site of the section.

Late-postglacial development of peat at Tlell – Port Clements and at Tow Hill on northeastern Graham Island appears to have resulted from induced paludification under the postthermal maximum relatively cool and wet climate, following the regression of the sea from this surface. Prior to this time estuarine conditions prevailed while isostatic readjustment and eustatic sea level change occurred. The abundance of charred peat at numerous levels in the sections indicates, however, that the climatic environment did not become sufficiently moist to prevent burning. This evidence suggests the possible antiquity at which man occupied the island, as he was probably responsible for starting these ancient fires.

Sea regression which began in the late glacial reached a state of equilibrium about the time of the thermal maximum according to the studies of Cloud (6) on the organic reefs of the tropical Pacific. Wave-cut benches, formed during this static period, are today five to six feet above the present level, and the subsequent change as eustatic lowering is attributed to renewed growth of ice on the land. The location of the Sandspit deposit nearest sea level and represented by the least accumulation of peat, compared to the two preceding, may have had its origin in conjunction with this most recent change in land–sea level relations. It also seems likely that offshore currents have operated to block the drainage and impound the water body. With more recent sea level lowering, the water table was also lowered and hydrarch succession favored.

It is regrettable that the Queen Charlottes are so poorly explored botanically. A comprehensive survey of the flora would be expected to uncover a high degree of endemism and would bolster the foregoing evidence for a long period of biotic isolation beginning prior to the last glaciation. It is clear that work on the insular flora as well as on additional peat sections is necessary before an unequivocal conclusion can be reached regarding the refugium problem. The location of a muskeg near Masset in which a depth of 7.5 meters was

penetrated during the time of this study is encouraging and may serve as a place of departure for future research. Since insufficient shafts were on hand to continue sampling deeper, the bottom of the deposit was not reached. A cursory examination of the pollen at 7.5 meters shows that the pine maximum is at this level.

Muskegs situated on unglaciated terrain and higher in elevation than the late-glacial sea level maximum are requisites for obtaining the oldest record of the past on the islands. Pollen and peat diagrams and radiocarbon-dated horizons for these deposits are the necessary keys to interpreting this record.

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THE PEACH REPLANT PROBLEM IN ONTARIO

I. SYMPTOMATOLOGY AND DISTRIBUTION[1]

By L. W. Koch[2]

Abstract

In Ontario, serious difficulties are frequently encountered in the establishment of young peach trees as replacements in old orchard sites. Symptoms vary from slight stunting to complete absence of growth. Causal factors described in conflicting reports in literature on the subject and information gained from examination of affected trees attest to the complexity of the problem. The failure of the replanted trees has been ascribed to the activity of insects and nematodes as well as to the effect of nutritional disturbances, toxic agents, and spray residues. The problem is being studied as a co-ordinated investigation by plant pathologists, entomologists, microbiologists, horticulturists, nematologists, and chemists. The account given here is introductory to a series of papers dealing with the various phases of the investigation and includes mainly a review of the literature and a description of the symptoms and effects of the disorder.

Introduction

For some years peach growers in southwestern Ontario have been aware of the increasing difficulty experienced in establishing young peach trees in old orchard sites. Although the difficulty was well known at the Harrow laboratory, it was not regarded as serious until growers, in requesting assistance, pointed out that in order to maintain economic acreage units they had already been forced to plant young peach trees on old orchard sites immediately after removal of the old trees. Their farming programs made it impractical to rotate peach orchards on their own farms, and the growers were unable to expand their acreage by the purchase of adjoining property. It was then decided to circulate a questionnaire through all peach-growing districts of Ontario in order to assess the extent and importance of the failure to establish new trees as replacements for old ones.

Of the replies received, 48% of those from the Niagara district and 65% of those from southwestern Ontario indicated that losses from replanting in old orchards were either moderate or great. Answers also revealed that difficulty occurred in areas where entire blocks were replanted as well as where only scattered trees were replaced. Analysis of the questionnaire took into consideration the fact that as earliest symptoms of the trouble are discernible on the underground portions of the trees, numerous cases of mild stunting are overlooked by all but the most careful observers. It was additionally apparent, then, that the problem is of primary economic importance in Ontario.

A review of the literature concerning the peach replant problem and related problems elsewhere, as well as a study of the results of numerous root examinations of affected trees, made it evident that the condition was highly complex and would necessitate a co-operative investigation in the fields of pathology,

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Contribution No. 1459 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] *Principal Pathologist, Science Service Laboratory, Harrow, Ontario.*

entomology, microbiology, chemistry, and horticulture. Furthermore, it seemed probable that such an approach might not only solve this particular problem but also furnish information of a highly fundamental nature in regard to similar problems, several of which have been of particular concern to the Harrow laboratory.

Symptoms and Observational Evidence

Aboveground symptoms of affected peach replants in Ontario are usually in the form of retarded growth, eventual stunting, and various degrees of intercostal chlorosis. Where the trouble occurs, its severity may vary from a slightly retarded growth, as compared with adjacent areas which have not grown peaches, to complete failure. The symptoms of greatest diagnostic value, however, are found on the roots, which show varying degrees of discoloration and necrosis. Critical examination invariably discloses brown lesions on otherwise white root laterals, and often the repeated rotting-off of the finer roots and the subsequent development of new ones results in a sort of witches'-broom on the current year's roots, especially towards the end of the growing season. This condition has been observed on all types of soil in which peaches are commonly grown in Ontario and, although it more commonly occurs within the first two years after removal of the old orchard, the effect is still observable approximately four years after removal of the old trees.

Another characteristic of the problem frequently observed both in Ontario and elsewhere is the satisfactory growth of such fruit trees as sour cherry and apricot in locations where replanted peaches have failed.

Occasionally the trouble is so severe that peach replants die. In such cases growth in the spring usually begins normally. After an interval varying from a week to several months, the new roots die back to their point of origin on the old root; the top then collapses and the tree dies. These dead or dying roots often show no microscopic evidence of invasion by bacteria or fungi.

Another striking feature is the earliness of symptoms on the root growth in the spring. Numerous root examinations on new trees of various ages and particularly on volunteer peach seedlings from germinating peach pits in old orchards have revealed that root discolorations and lesions are frequently found on young root laterals as early as 24 hr. after emergence from the parent root tissues. In numerous cases microscopic examination of such rootlets has indicated that nematodes are the cause of the lesion but in many others individual discolored or dead cells or groups of cells were found entirely devoid of visible organisms.

Review of Literature

The cause of the failure of peach replants in old orchard soils has been variously ascribed to insects, nutritional disturbances, toxic agents, spray residues, and nematodes. A considerable number of such reports from

different countries concerning not only peaches but other fruits, such as apples, cherries, citrus fruits, and figs, demonstrate that the replant problem is widespread.

With a few exceptions only comparatively recent literature will be referred to in this article.

Contributing or Causal Factors

Aphids

Cutright (7) reported that the black peach aphis *Anuraphis persicae-niger* was a limiting factor in the starting of young trees at certain places in Ohio. In a later publication the same author (8) reported that other more important factors than the black peach aphis were probably responsible for the decline of replanted peaches.

Nutritional Disturbances

Upshall and Ruhnke (33) in Ontario reported striking differences in growth of Mazzard cherry, Myrobalan plum, French pear, and Mahaleb cherry planted in soil formerly occupied by a peach orchard for a period of six years. The areas of weak growth coincided almost exactly with the former locations of the peach trees in the original orchard. Examination of the soil failed to show significant differences in the fertility status of former sites and intersites. The authors concluded that decreased nitrification resulting from the decomposition of the peach roots in the tree areas, in addition to the lowered supply of mineral nutrients (phosphorus and potassium), may have been the factors responsible for the retarded growth first noted as typical of the tree areas. They concluded further that residual effects from occupation of land by peach trees for a period as short as six years might continue to cause retarded growth of subsequently planted fruit-tree stocks after at least a similar period of time.

Hewetson (12) reported on his investigation of the peach replanting problem in southern Pennsylvania. His experiments were designed to evaluate the various hypotheses suggested by previous workers, especially the one respecting toxicity by Proebsting and Gilmore (26). Peach trees were replanted in former peach orchard sites where soils were given various treatments with fungicides and fertilizers. Records were obtained by measuring the trunk diameter and terminal growth of the trees and the length, width, and dry weight of the leaves and by determining the nitrogen and chlorophyll content of the leaves. Hewetson concluded that the liquid fertilizer he employed had the capacity to produce strong, vigorous peach trees superior to those produced with either manure or a 10 : 10 : 10 fertilizer. He further stated that if trees could be made to grow vigorously during their first year in the orchard they might be expected to continue satisfactory growth in subsequent years.

Toxic Agents

The possibility that soil toxins are responsible for the unsatisfactory growth of second and later plantings of the same crop, including fruit trees on the same soil, has intrigued workers for many years. The reports of Massey (21)

and Strong (31) on walnuts, Schreiner (28) and Schreiner and Reed (29) on wheat, Proebsting (24, 25) and Proebsting and Gilmore (10, 26) on peaches, Benedict (2) and Myers and Anderson (22) on brome grass, and Bonner and Galston (3, 4) on guayule strongly suggest a toxin or toxins as a possible explanation for the difficulty so often experienced in replanting old sites to the same plant species.

Proebsting and Gilmore (26) reported on the relation of peach root toxicity to the re-establishment of peach orchards in California. Their survey of the important peach districts in that state indicated that results in the replanted orchards varied from complete success to the failure of many of the trees to grow. They were unable to obtain adequate or consistent response with the use of any of the basic nutrients. They also did not obtain any stimulation of growth from the use of Mn, Zn, Cu, vitamin B1, indole – butyric acid, or indole – propionic acid in various amounts and combinations. Peat moss proved to be beneficial in some cases. The presence of crown gall (*Agrobacterium tumefaciens*) and oak root fungus (*Armillaria mellea*) was considered to be an important factor in certain cases but as these diseases can be readily diagnosed they would not be confused with the more general peach replant problem. These authors found that peach roots added to virgin soil inhibited growth of peach seedlings. In sand culture, the root bark, but not the wood, was found to be toxic to peach seedlings. The alcohol extract of the bark, but not the extract of the wood, proved to be toxic. The residue from alcoholic extraction of the bark was not toxic. The specific compounds involved were not identified.

Havis and Gilkeson (11), in their studies of the peach replant problem reported in 1947, also considered the possibility that toxic factors are involved. They grew Lovell peach seedlings budded to Elberta in sand in earthenware crocks. The seedlings were fed by means of nutrient solutions. Leachates of roots and bark added to nutrient solution were pumped into the crocks three times daily for a period of seven months. The root bark was treated variously and amounts up to 400 gm. of chopped peach roots were added in each case. These workers concluded from their experiments that there was no evidence of any toxic substance in peach roots or in peach leachate that adversely affects the growth of young peach trees in high-nutrient sand culture.

In further studies of the effect of peach root residues Proebsting (24) in 1949 reported erratic results. He concluded that unaltered root residues could not be the primary cause of the trouble and felt that he had also eliminated parasites and nutrition as primary causal factors. The following hypothesis was then offered as an explanation for the condition.

"If root residues are acted upon by one set of soil organisms to produce substances toxic to peach trees while other sets of soil organisms decompose residues in such a way that nontoxic products are produced, the observed facts can be fitted in. The high degree of specificity noted would be tied to peach root residues as a source of the material produced, while the unpredictable

behavior would be accounted for by the presence or absence of the toxin-producing soil organisms." No experimental evidence was presented to support or reject the hypothesis.

In 1950 Proebsting (25) reported on a case history of a "peach replant" situation in California. In this study, which covered a period of six years, observations were made on growth and other characters of eight different kinds of fruit trees replanted in soil where the same or different fruit varieties had grown in preceding years. He found that the peach-after-peach group not only grew less rapidly during the first year but grew even more slowly in subsequent years. On the contrary, the peach-after-apple trees made normal growth each year. He concluded that certain characteristics stand out in the peach replant problem and are basic to any hypothesis of explanation. One is the high degree of specificity so clearly manifested by the differences in vigor of different kinds of fruit trees replanted in former peach orchard sites. Such relationships were evident both in experimental plantings and in commercial blocks.

The second characteristic noted by Proebsting is the high degree of variability found in both field and pot tests, and the lack of any method known for prediction of the behavior of a particular block of peach replants. Proebsting also pointed out that no clear-cut symptoms other than growth rate are evident in orchards showing intermediate effect, and that numerous samples submitted to plant pathologists and entomologists failed to show parasites. Furthermore, additions of nutrients failed to correct the condition completely. The hypothesis pertaining to cause, outlined in his previous paper (24), is repeated and he further concluded that this explanation might also account for the benefit often secured by soil fumigation. He explained that the partial or complete failure of this method of control may be due to the fact that partial decomposition and production of toxin may have occurred before soil fumigation, thus leaving toxic material to produce some replant effect. This assumption could account for the complete or the partial failure of this method of control.

Gilmore in California, in correspondence, stated that although there are present in peach root residues substances capable of reducing the growth rate of laboratory-grown peach seedlings, he was unable to demonstrate response to root residues in pot culture or in the field.

Nematodes

In recent years nematodes have been suspected of causing peach replant failures in certain areas. Johanson (13) reported a case in Connecticut. In this instance, specimens were submitted to Steiner at Washington who reported them to be infested with meadow nematodes, *Pratylenchus* spp., which attacked the root tips and finally induced a witches'-broom root system.

In a subsequent survey of peach orchards in Connecticut, Johanson examined samples from 12 widely distributed peach orchards. In every instance either a species of *Pratylenchus* or a species of *Criconemoides* or both were found to be present in the roots.

As a result of examination of roots of declining peach trees Chitwood (5) suspected that nematodes of the genus *Criconemoides* were a factor in the decline and replanting of peach orchards in Maryland.

Day and Serr (9) and others (32) reported on a study of the comparative resistance of rootstocks of fruit and nut trees to attack by a root lesion nematode in California. They concluded that under the conditions of their test common apricot, apple, pear, and quince stocks were resistant to attack by *Pratylenchus vulnus*. Plum and peach stocks varied in their susceptibility but Macedonian Wild, Etter's Best, and Bruce plums were highly resistant and Bokhara and Yunnan 55885 peaches were apparently at least partially resistant.

The nematode *Belonolaimus gracilis* was found by the same authors to be associated with decline in peach orchards near Holland, Virginia. They concluded that this species may do considerable damage to the roots since it is ectoparasitic and migratory.

Panagrolaimus spp. were also found inside decaying peach roots and coiled within apparently living cells where no decay had set in. Day and Serr were uncertain whether or not these organisms are primary. They also stated that *Criconemoides simile* and *Belonolaimus gracilis* will eventually have to be given some consideration.

In the course of investigating the resistance of six peach root stocks to two of the principal species of peach root knot nematodes, Chitwood, Specht, and Havis (6) reported their observations concerning other nematodes. Species of *Criconemoides*, *Xiphinema*, and *Belonolaimus* were in each case found to be the predominant nematode in the vicinity of the roots of certain declining trees in the sandy coastal plains area. *Criconemoides simile* was screened out of soils from the Eastern Shore of Maryland, and Lovell variety peach seedlings were inoculated with it. These seedlings showed chlorosis and a significant reduction in growth, with leaf-dropping from the base upwards. *Xiphinema americanum* was found to be the predominant nematode associated with declining peach trees in certain instances in Maryland. No experimental evidence of its pathogenicity is available.

These authors (6) stated that, in addition to causing direct effects on the growth of trees, nematodes evidently may affect plant growth through their influences on chemical composition of plants. From infection experiments with *Meloidogyne javanica* and *M. incognita*, and subsequent spectrochemical leaf analyses, they concluded that chlorosis in peach appears to be due to deficiency of magnesium or iron or both. Either or both may be absorbed differentially by *M. incognita* and *M. javanica*. Moderate numbers of these two nematodes sometimes cause significant increases in peach growth. Stimulation of growth of peach seedlings by the nematodes in question was associated with greater accumulated magnesium and calcium in the leaves than when no nematode injury occurred. Of all nutrient elements determined in the leaves, potassium was unique in that it usually was present in increased amounts regardless of special treatment against nematodes or of seedling variety.

Miscellaneous

In addition to the various causal factors reported above, certain other references to the replant problem are of interest. Johanson (14) reported that, of various soil disinfectants used to control the condition in old peach orchard sites, the only successful one was formaldehyde. The New Jersey Experiment Station (23) in 1937 reported that during late spring moisture conditions in the *B* horizon were likely to be unfavorable for replanting peach orchards. According to Proebsting and Gilmore (26), Wallace considered that the poor results of replanted peaches were due to continued cultivation and that unsatisfactory physical condition of the soil was a major factor. Verner (34) regarded the spray residue, arsenic, as a causal factor in the poor growth of replanted fruit trees. In 1939 Klaus (15) presented a comprehensive review of literature, especially that in German, on the problem of replanting fruit trees but included few references to peaches. More recently, Savage (27) reported an investigation in Georgia to determine the parts played by nutrition and toxic residues in the peach replant problem. He found no relationship between either nutritional treatment or toxic residues and injury to the trees. On the other hand, he found a close relationship between the incidence of a root rot caused by a species of *Clitocybe* and the occurrence of the peach replant trouble. He concluded that in Georgia *Clitocybe* root rot is "apparently a major cause of heavy tree losses when old orchard sites are replanted."

Shannon and Christ (30) reported tests in connection with the peach replant problem in two one-acre plots in New Jersey. In addition to establishing a basic fertilizer program in these plots they experimented with various mulches, minor elements, and soil fumigants. Although no significant differences in tree growth or yield resulted from any of the mulch or minor element treatments, significant improvement in tree growth did result from the use of the soil fumigant D-D. Chemical analyses of foliage failed to disclose any relationship between nutrient levels and tree growth. The presence of parasitic nematodes in one of their experimental orchards led the authors to conclude that the growth response resulting from soil fumigation may have been due to the elimination of the nematodes. However, no data as to the species involved or their abundance were reported.

"Slow Decline of Citrus" as Possibly Related to Peach Replant Failure

A condition in the citrus groves of California known as "slow decline of citrus" or as the effect of "citrus sick soil" is strikingly similar in nature to the peach replant problem and has been investigated intensively by Martin *et al.* (16, 17, 18, 19, 20). Martin (16, 17) reported that the treatment of old citrus and noncitrus soils by steam under pressure or by leaching, or with various soil fumigants, fungicides, or insecticides, followed by cropping with sour or sweet orange seedlings, noticeably affected the nature of the fungus population of the soil. The observations of Martin and his co-workers suggested that a detrimental soil population was partly responsible for poor growth of citrus seedlings in old citrus soils, and that the change in microbial

balance resulting from partial soil sterilization may be one factor involved in the improvement in plant growth. On the other hand, the fact that the fungus population was completely changed in many treated soils and the growth of orange seedlings still was not so good as in the noncitrus soils, although better than in the controls, suggested to these workers that factors other than the soil fungi also were partly responsible for reduced growth in old citrus soils.

Martin and Batchelor (19) also reported that ordinary fertilization did not rectify the condition in citrus, and heavy treatment of the soil with such fumigants as carbon disulphide, D-D, chloropicrin, and ethylene dichloride usually improved growth of citrus seedlings, and in some cases more than doubled it, but did not increase growth to a point comparable to that in fumigated or untreated noncitrus soil. Then, on the assumption that a toxic substance might be involved with "slow decline" of citrus, Martin conducted various leaching experiments. He concluded that his results supported the hypothesis that there accumulated, in addition to detrimental organisms, a toxic material in soil which is continuously cropped to citrus for long periods. This material presumably is toxic to citrus but not to other plants such as avocados and tomatoes; it is either only slightly soluble in water or adsorbed by the soil and held against leaching with distilled water; it is apparently not adsorbed as a replaceable base; and it is apparently destroyed by, or is soluble in, relatively strong acid or base solutions.

As a result of further investigation Martin (16) concluded that the stimulation of citrus plants grown in fumigated, old citrus soils cannot be attributed to increased availability although that possibility definitely exists where other crops and certain soil types are involved.

From a study of the effect of soil fumigation on growth and chemical composition of citrus plants Martin *et al.* (18) found that citrus seedlings grown in old citrus and noncitrus soils were not significantly different in chemical composition. All plants contained adequate amounts of most nutrient elements normally found in healthy citrus plants. They concluded that destruction of parasitic and possibly other detrimental soil organisms best explains the growth-stimulating effect of soil fumigation on citrus replants.

In a recent study Martin *et al.* (20) reported that growth of citrus may be apparently affected by variation in exchangeable K, Na, Ca, and H and by excess lime, and that favorable exchangeable cation ratios are more critical for a second planting than for the original crop. With the exception of increased leaf manganese in some tests the few changes resulting from fumigation did not change the level of any nutrient from a deficiency to a sufficiency status. They concluded that as far as the soils in their study were concerned the changes effected in the status of the nutrient elements in old citrus soils by fumigation do not account for the increase in growth of the citrus plants (1, 20).

Discussion

It is apparent from the foregoing review of literature that, potentially, trouble may develop wherever peaches are replanted in old peach orchard sites

on this continent. It is also apparent that wherever this trouble occurs it exhibits the same general pattern of host specificity and symptom complex. On the other hand, the conclusions and theories pertaining to causal factors are somewhat conflicting. The explanation for this situation may possibly lie in the fact that several problems may be involved rather than a single one, the relative importance of each varying according to local conditions. A general appraisal of the various investigations dealing with peach replant failure suggests that in most cases the approach to the problem has been too limited, usually only one or a few phases being dealt with. For a problem so complex such an approach almost inevitably leads to interpretations and conclusions only partially correct in terms of the problem as a whole. For instance, in the case of those investigators who concluded that the peach replant problem is a nutritional disorder the possible role of pathogenic organisms and certain other factors was nearly always ignored or given inadequate consideration.

In the main, the workers in California have contributed more to date than others concerning the peach replant problem, notwithstanding the fact that the results of their efforts remain somewhat inconclusive. Proebsting and Gilmore (24, 25, 26), after ruling out pathogenic organisms or nutritional factors as causal agents in the failure of replanted peach orchards to grow satisfactorily, demonstrated that when peach roots were incorporated into sand in greenhouse experiments they were inhibitory to the growth of young peach trees. A similar effect was obtained by using alcohol extracts of peach roots, the alcohol-insoluble residue being nontoxic to peach trees. Thus, it was demonstrated that the roots of the peach tree contain an inhibitor of growth, although it was not shown that the substance concerned actually escapes into the soil and is active under field conditions. On the other hand, Havis and Gilkeson (11) in California found no evidence at all of toxic substances either in peach roots or in peach leachates to account for the disorder.

Certain other workers, such as Johanson (13) in Connecticut, Chitwood (5), and others (6, 9), have presented evidence that nematodes are an important factor in the decline of peaches grown after peach in an orchard rotation in the areas concerned. However, lack of statistics in connection with the occurrence of nematodes on peaches makes it impossible to evaluate their real importance.

Certainly, the greatly improved tree growth resulting from the application of soil fumigants such as ethylene dibromide, propene–propane mixture, and methyl bromide to old orchard soils in Ontario and other areas strongly supports the theory that nematodes may be an important factor underlying the peach replant problem. It should also be mentioned, however, that the variability in response to soil fumigation in these experiments suggested that either the factors causing peach replant failure may not be the same in all orchards or the concentration of these causal factors may vary considerably from orchard to orchard.

Thus, it seems obvious from a review of the literature concerning the peach replant problem and from preliminary observations and examinations of affected root systems that anything less than a combined and co-ordinated approach in the fields of horticulture, microbiology, chemistry, plant pathology, and entomology would be likely to result in disclosing only partial truths regarding causal factors, and that causal factors and their relative importance must be thoroughly understood so that a lasting or consistently beneficial control program may be achieved.

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THE PEACH REPLANT PROBLEM IN ONTARIO

II. TOXIC SUBSTANCES FROM MICROBIAL DECOMPOSITION PRODUCTS OF PEACH ROOT RESIDUES[1]

By Z. A. Patrick[2]

Abstract

Experiments were carried out to evaluate the importance of soil toxins in the peach replant problem and to determine whether the substances produced by the interaction of microorganisms occurring in old peach orchard soils and peach root residues were toxic to living peach roots. A rapid and accurate indication as to whether or not these substances were toxic was obtained by determining their effect on the respiration of excised peach root tips. It was demonstrated that substances which inhibit the respiration of excised peach root tips are produced when peach root residues, and also, chemically pure amygdalin, are acted on by certain microorganisms occurring in old peach orchard soils. Such substances were not produced when the soils were autoclaved before amygdalin was added or when other soils were used in which no breakdown of amygdalin had occurred. Again no such inhibiting substances were produced when other root residues, such as sour cherry, tobacco, or pepper roots, were added to these soils in place of peach root residues or amygdalin. The inhibiting substances were readily obtained by extraction with water and they proved to be highly active physiologic agents; the inhibiting effect on the respiration of peach root tips, which ranged from 40 to 90%, could be detected after tips had been less than half an hour in the toxic leachates. In addition to inhibiting respiration these substances also induced darkening and finally the necrosis of meristematic cells. All of these effects proved to be irreversible after tips had been five hours in the toxic leachates. The root tips were apparently killed after this time. Parallel experiments were carried out also with pure amygdalin, and similar inhibition of respiration and injurious effects on meristematic cells were obtained with microbial degradation products of either amygdalin or peach root residues. Water solutions of amygdalin, emulsin, amygdalin and emulsin combined, and various benzaldehyde dilutions were also tested. Inhibition of respiration and darkening of apical meristems were only obtained, however, in solutions where amygdalin was hydrolyzed by emulsin and in the various concentrations of benzaldehyde in water. They were not obtained in the separate solutions of either the glycoside or the enzyme. It was concluded, therefore, that microbial action on the amygdalin fraction of peach roots is mainly responsible for the toxic factor frequently encountered in old peach orchard soils.

Introduction

The literature pertaining to the peach replant problem has been adequately reviewed in the first paper of this series (10) and from this survey it is apparent that there is a great deal of controversy as to the primary causal factor responsible for this condition. From the variable and contradictory results one must conclude that the peach replant problem is a complex one for which no single factor is responsible. The roles played by toxins, nematodes, fungi, bacteria, soil fertility, and other factors found associated with the detrimental soil condition must all be considered before one can hope to arrive at any conclusion as to the primary cause.

Because of the failure to isolate specific pathogenic organisms many investigators concluded (12, 13) that toxic substances had to be present in the

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Contribution No. 1460 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] *Associate Plant Pathologist, Science Service Laboratory, Harrow, Ontario.*

soil where peaches have grown for some time and that these toxic substances might be partly responsible for the poor growth of the peach replants which follow. It has also been observed (15) that inhibition of growth of the new peach replant is much more severe in the area of the old peach tree site. Of the peach replants which die most of them are those planted in locations from which an old tree has recently been removed. This detrimental soil condition in the area of the old site is maintained in many cases even after soil fumigation with such fumigants as methyl bromide or ethylene dibromide. After soil fumigation, peach replants planted in intersite soil, that is soil in areas three feet or more away from the site, show a much improved growth while those planted in site soil show only a slight improvement (see Fig. 4). The site–intersite effect, however, is considerably modified in two to three years, after which time usually no differences between the two areas are apparent.

Although a toxic principle has been suspected for some time it has been very difficult to prove its existence in controlled laboratory or greenhouse experiments. Proebsting and Gilmore (13) for example, presented evidence to show that a toxic factor is found in peach root bark residue. They obtained stunting by the addition of the water extract of peach root bark to sand cultures of growing peach seedlings. Similar stunting was obtained when they added amygdalin and emulsin solutions to the growing peach seedlings and no stunting when they used amygdalin or emulsin alone. Havis and Gilkeson (9) on the other hand failed to reproduce the stunting demonstrated by Proebsting and Gilmore even when they used as much as 400 gm. of peach root residue.

Besides the usual chemical constituents found in any woody root, peach root contains a cyanophoric β-glycoside, amygdalin, or, if in a partially reduced state, prunasin (2). These glycosides in themselves are not toxic but if acted on by emulsin enzymes, the β-glucosidases, the amygdalin is broken down into glucose, benzaldehyde, and hydrogen cyanide. Since benzaldehyde oxidizes readily in the presence of air (7), some of it is probably converted to benzoic acid. The enzyme which acts on amygdalin is also present in the living peach root tissues but separated from the amygdalin (2). When the cells are ruptured by any lesion-producing agencies such as nematodes, fungi, or insects, the two substances are brought together and the above-mentioned products are released. This is readily demonstrated when fresh peach roots are crushed and the smell of benzaldehyde and cyanide is noted. Other sources of the emulsin enzymes are some soil microorganisms which can utilize the amygdalin as a nutrient. The microorganisms capable of utilizing this substrate will be favored in areas where the glycoside occurs and they tend to build up in such areas as long as the raw material is available (4). When a peach tree is removed most of the root system is left in the ground and consequently in old peach orchard soils, especially in areas of the old tree sites, there is a large supply of this raw material. As long as the glycoside is available where there are organisms capable of utilizing it the toxic breakdown products mentioned above could be produced. In addition

to the amygdalin contained in the roots and root residues which are left in the soil when the tree is removed, there is an active synthesis of the glycoside and the appropriate enzyme throughout the life of the tree. Any injury to the root tissue cells containing these substances will bring the two together again and allow the toxic substances to be released. There is still the possibility of other sources of toxic substances being produced in peach soils because of the predominance there of amygdalin-utilizing organisms. The resulting shift from the normal heterogeneous microflora to one containing high numbers of organisms having essentially similar requirements and capabilities with respect to the amygdalin substrate might give rise to other toxic or antibiotic substances being synthesized by this special microbial flora.

The above-mentioned toxin-producing possibilities have no doubt been foreseen by other investigators (12, 13) working on this problem and although a soil toxin hypothesis has been put forward a number of times very little experimental evidence has been presented to establish the relative importance of soil toxins in the problem. Thus it is not known whether special groups of microorganisms are necessary for toxin production or whether the toxins are due entirely to the presence in the peach roots of the glycoside amygdalin.

The purpose of the studies described here was to investigate further the relative importance of toxic soil substances in the peach replant problem by determining how these toxins arise, whether or not microorganisms are necessary for their production, and whether they are due entirely to the presence of amygdalin, and by noting the specific effects of the toxins on the peach root system.

It should, however, be stressed that other contributing factors are recognized as being partly responsible for the difficulty of re-establishing peach trees in soil which previously supported a peach orchard. Because the problem involves so many different factors and interrelationships, however, it is of primary importance to study separately each factor that may contribute to it. Other contributing factors will be evaluated in later studies from this laboratory.

Materials and Methods

For the first series of experiments the materials used were those normally encountered in a replant-problem peach orchard, namely soils obtained from the site and intersite containing the microorganism normally found in each of the areas. To these soils was added peach root residue in various amounts and enough water to bring the moisture content of the soil approximately to field level.

The soil used was of the Fox sandy loam type, fresh samples being obtained from a 14-year-old peach orchard at Harrow Science Service Laboratory immediately before each experiment. At the beginning of this study a block of old peach trees was replaced by young peach trees, and in the replants stunting and other characteristics of the peach replant problem developed. Soil was obtained from two areas in the orchard: the area from which the old

tree had been removed, which is referred to as the site area, and the space between the rows of trees about 10 ft. from the site area, referred to as the intersite area. In many cases soil was taken close to an old peach tree which was still standing and this is also referred to as site area soil. The soil was obtained at root depth from 12 to 36 in. from around the roots and in the most concentrated root area, up to 3 ft. from the old site; it was mixed thoroughly and this composite soil sample was used in the experiments as site area soil. The soil from the intersite area was obtained from a similar depth but about 10 ft. away from the site. While these samples were being collected many living and dead roots were encountered in the site area but very few peach roots were seen in the intersite. The soil samples were passed through a 20-mesh screen to remove all pieces of roots, stones, and other foreign materials. For other experiments a soil similar to the peach soil, but which had no previous peach history, was also used, and in addition, different soil types including a muck soil with a high organic content and a compost soil regularly used in the greenhouse.

The peach root residue used in these experiments was obtained from roots of 2-year-old Lovell peach seedlings grown in typical peach soil with no previous peach history. The trees were dug, the roots cut off and dried. To avoid the possible loss of water-soluble amygdalin the roots were not washed but adhering soil was shaken or brushed off. The smaller roots were ground to a coarse powder. The bark was removed from the larger roots and this was also ground to a coarse powder to become the peach root residue referred to in the experiments. The woody parts of the large roots were not used. The amygdalin used in many of the later experiments was the chemically pure compound obtained from a drug supply house.

The toxic extracts were obtained in the following way. To Erlenmeyer flasks containing fresh soil samples taken from the various areas were added known amounts, from 1% to 5% of the flask soil content by weight, of peach root residue. The flasks were then incubated for varying periods of time, the water content of the soil being kept at approximately 60% of the moisture-holding capacity. Most active toxic extracts, when peach root residue was added to the soil, were produced when the soil and residue were incubated at 25° C. for three to six days, whereas in experiments where peach root residue was replaced by amygdalin, four to eight days' incubation was necessary. After the various incubation periods crude preparations of the toxic substances which were produced in the flasks were obtained by extraction of the flask contents with water, using 1 cc. of water for each gram of soil. The extraction was continued for half an hour with intermittent shaking every 10 min., after which the supernatant was filtered through several layers of cheesecloth and often also through a Seitz filter before being used in the tests.

Since HCN is one of the substances produced when amygdalin is decomposed, the ability of the microorganisms in the various soils to break down the amygdalin in the peachroot residue was followed by testing for HCN by the "sodium carbonate picric acid paper test". The same test was used for

following the comparative rate of breakdown of the chemically pure amygdalin in the various soils. The sodium carbonate picric acid paper (hereafter referred to as Na picrate paper) was prepared according to the method described in the *Official methods of analysis of the Association of Official Agricultural Chemists* (3). If HCN is present, the yellow Na picrate paper gradually turns orange and then brick red. The test is delicate and the rapidity of change in color depends upon the quantity of free HCN present, hence on the rate of amygdalin breakdown. The amount of cyanide produced in a certain time interval is therefore a reflection of the microbial degradation of the glycoside in that soil type and is assessed in terms of the intensity of the red color of the Na picrate paper.

The rates of amygdalin breakdown in soil samples obtained from the Science Service peach orchard (site and intersite areas), from the sour cherry orchard, from a muck soil, and from a compost soil were followed by the Na picrate test described above. Erlenmeyer flasks each containing equal quantities of the various soils and peach root residues were prepared in the manner indicated. Each flask was plugged very carefully so that each plug was of equal firmness in order to allow identical aeration. In later experiments to avoid aeration errors each flask was corked with a rubber stopper. Some of the soil samples were partially sterilized by autoclaving at 15 lb. for 20 min. The flasks were then incubated at 25° C. for 1 to 10 days and the rate of amygdalin breakdown was determined every 24 hr. by testing the rate of CN production.

The peach seedlings and peach root tips used in this study were obtained from Lovell seeds which had been afterripened for 85 to 110 days at 3° C. in moist vermiculite. The afterripened seeds were then surface-sterilized and planted in flats containing sterile vermiculite. Four to seven days after planting, a single sturdy radicle 20 to 30 mm. long, similar to those shown in Fig. 9, was put out from each seed. This was followed, 9 to 12 days after planting, by 8 to 15 secondary roots approximately 1 mm. in diameter and 20 to 35 mm. long. In some of the early experiments only the tips of the radicles were used, but this required too many peach seeds, so in all later experiments secondary roots, which were much more plentiful, were used. These were found to be as suitable and appeared to be more sensitive to the toxic extracts. Growing tips 8 mm. in length were cut off and placed in distilled water. Variations due to root hairs were not encountered because no root hairs occurred at less than 25 mm. from the tips. The root tips were cut off immediately before each series of experiments and a fresh sample of root tips, taken from seeds planted at the same time and growing under identical conditions, was used for each new run. The roots were rinsed in distilled water and blotted; then equal numbers selected at random were transferred to Warburg reaction vessels containing the various test filtrates.

The method used for testing the deleterious effects of the microbial breakdown products of either peach root residue or of amygdalin on living peach roots will be described in more detail later. Generally the test for toxicity

was based on the percentage of inhibition of respiration of peach root tips placed in the various toxic preparations as compared to the respiration of idential root tips in filtrates considered to be nontoxic. The respiration studies were carried out in the Warburg respirometer following the usual manometric procedures (14). The direct method of Warburg was used to determine the oxygen uptake of peach root tips in the various test filtrates.

The relative abundance and types of soil microorganisms present in the peach orchard soil collected from the site or intersite areas and capable of utilizing peach root residue or amygdalin were determined by the regular soil dilution plating methods (8). Either peach root residue or amygdalin incorporated into an agar medium as the only nutrient source was used for the plating medium. The organisms capable of utilizing these substrates were counted and then isolated for further studies.

Observations and Results

Decomposition of Peach Root Residue in Various Soils

If soil toxins are partly responsible for peach replant failure, some of these toxic components should be produced by the action of the characteristic microflora of old peach orchard soils on peach root residue in the presence of water. Since the decomposed amygdalin fraction of peach root residue comprises potentially toxic products, experiments were first carried out to see whether or not and how rapidly soil microorganisms are capable of breaking down the amygdalin naturally occurring in peach root residues. It has been observed that the new peach replants are much more severely stunted when they are planted in the old site. It was decided therefore to see whether there are differences in the rate of amygdalin breakdown between soil samples obtained from the site and the intersite areas. It was also decided to use other soil types to see if any differences existed in their capability of breaking down amygdalin and hence whether there were likely to be differences in the types of microorganisms present in them. These results are presented in Table I.

The results summarized in Table I show that amygdalin decomposition in the soil can be followed readily by the qualitative test. Although there are some differences in the relative rates of amygdalin decomposition exhibited by the various soil samples it should be stressed that these can be interpreted at this time only as suggestive trends. Without additional evidence it is impossible to say whether or not the amygdalin decomposition is a reflection of the microbiological situation as it exists in a peach orchard having a replant problem. Since we are dealing with a system as heterogeneous, variable, and dynamic as the soil microbial flora, many more soil samples obtained from other peach orchards should be tested and the CN produced should be analyzed on a more quantitative basis before any conclusions can be drawn as to the relative rates of amygdalin breakdown. It can be concluded, however, that the amygdalin present in peach root residue is broken down by soil microorganisms. When the microorganisms are eliminated by autoclaving no

TABLE I

RELATIVE ABILITY OF SOIL MICROORGANISMS IN VARIOUS SOILS TO DECOMPOSE THE AMYGDALIN IN PEACH ROOT RESIDUES

2% Peach root residues added to fresh soil samples obtained from:	Test for CN after incubation at 25° C. for:			
	24 hr.	48 hr.	96 hr.	240 hr.
Old peach site area (soil under stump and around roots)	++++*	++++	++++	++
Intersite area (10 ft. from site)	++	+++	+++	++
Old site area (autoclaved)	---	---	---	-
Sour cherry orchard	++	+++	+++	++
Compost soil	---	---	---	+
Muck soil	---	---	---	+

* *Amount of CN produced determined by the intensity of red color of the Na picrate paper ½ hour in the reaction flask:*

++++ *Intense red color—strong CN production.*
+++; ++ *Decreasing intensity of red color—diminishing amounts of CN produced.*
\+ *Pale orange color—weak CN production.*
--- *Yellow color of Na picrate paper—no CN produced.*

breakdown of amygdalin occurs if the specific enzyme present in the peach root cells is also deactivated. Amygdalin breakdown begins more rapidly in soil from the site than from the intersite area. This may be a reflection of the presence in this area of a higher proportion of microorganisms which are capable of utilizing the β-glycoside as their nutrient source and which are not very sensitive to the toxic substances produced. Many of the microorganisms occurring in soil obtained from a sour cherry orchard are also capable of utilizing amygdalin. No test for CN was obtained with the compost or muck soils until 10 days of incubation when a weak positive test was obtained. Soil samples differ in their relative ability to break down the β-glycoside and some variations from the results presented were encountered. The tests were carried out on 37 different occasions starting in March and continuing through summer and fall until early January. Each time fresh soil samples were used, but in all cases they were obtained from the same general areas shown in Table I. On 26 occasions the results were essentially those summarized in Table I. Eight times no definite trends in the rates in site, intersite, and sour cherry soils were noted and on three occasions the results were reversed. In each case, however, there was no amygdalin breakdown in the autoclaved soil and only retarded breakdown in muck or compost soils.

Decomposition of Chemically Pure Amygdalin in Various Soils

To determine whether or not chemically pure amygdalin is decomposed as readily as that occurring naturally in peach roots, a parallel series of experiments was carried out at the same time as the above series except that 0.25% chemically pure amygdalin was substituted for peach root residue. The results obtained were essentially similar to those obtained with peach root

residue but were more convincing because use of the pure chemical eliminated the possibility of introducing into the various soil samples specific microorganisms occurring with the peach root residue or amygdalin in different amounts or in various stages of breakdown. The differences in the rates of amygdalin breakdown were more pronounced in the soil samples taken from site and intersite areas and from areas where peaches had not been grown than in the experiments where peach root residues were used. The initial lag period between the time that the amygdalin was introduced into the flask of soil and the time required for active CN production varied widely between soils from various sources and locations. After 15 hr. incubation, only the soil obtained from the old peach site area showed a strong test for CN. There had thus been a rapid amygdalin breakdown here and consequently most of the microorganisms occurring in this area were capable of utilizing this substrate. In soil from the intersite only a weak test for CN was obtained in most cases; thus after 15 hours' incubation very little amygdalin breakdown had occurred in the soil from this area and only after a build-up period of three to six days was a strong positive test for CN obtained. Again no amygdalin breakdown occurred in autoclaved soil, and cyanide could not be detected in compost or muck soils even after 10 days' incubation.

Toxic Effects of the Breakdown Products of Peach Root Residue on Living Peach Roots

(*a*) *Effect of Microbial Breakdown Products of Peach Root Residue or of Amygdalin on Respiration of Excised Peach Root Tips*

In the first series of experiments it was shown that the amygdalin naturally occurring in peach root residues as well as chemically pure amygdalin, can be readily decomposed by some of the microorganisms found in old peach orchard soils and that no breakdown takes place if these microorganisms are eliminated. Since one of the breakdown products was shown to be HCN, it was assumed that benzaldehyde and glucose, the other components of amygdalin, were also present (2, 7). Some of the benzaldehyde, because of its instability in air (7), would probably be oxidized to benzoic acid and this aromatic acid might also be present. The next series of experiments were carried out to determine whether some of the decomposition products mentioned, and possible other toxic substances synthesized by the microorganisms utilizing amygdalin or its components, could be extracted with water and if such leachates would produce any deleterious effects on living peach roots.

The toxic quality of peach root residues is usually demonstrated through the inhibition of growth of peach seedlings after the addition to the soil of such a residue or its extracts. Contradictory results obtained by different investigators (9, 13), have shown that this type of test is very unreliable, probably because differences can be detected only after an extended period of time, from 30 days to 7 months. It is apparent therefore, that a method which would demonstrate rapidly, and preferably at cell level, whether or

not these soil extracts were toxic to peaches would be much more valuable. Since respiration is of basic importance to the health of the individual (1, 5), it was hoped that any soil toxin would act on some of the many steps in the respiratory process and be reflected by a change in the respiration rate of the actively respiring meristematic region of the root. Consequently, in the toxicity tests used in these studies, the oxygen uptake of excised peach root tips placed in water leachates of soils containing microbial breakdown products of peach root residue was compared with oxygen uptake of similar root tips in water leachates of similar soils where no peach root residue was added or where no amygdalin breakdown had taken place. These tests were carried out as follows.

To Erlenmeyer flasks containing fresh samples of soil from an old peach orchard were added in proportions of 1%, 2%, or 5% by weight of peach root residue or, when amygdalin was used, 0.25% of the chemically pure compound. The flasks were plugged and incubated at 25° C. After from three to six days' incubation, when a strongly positive CN test indicated that active microbial degradation of amygdalin was taking place, 1 ml. of water for each gram of soil was added to each flask. The flasks were shaken intermittently for half an hour and the contents allowed to settle. The supernatant liquid was then decanted and filtered through several layers of fine cheesecloth. In some instances the extracts were also filtered through a Seitz filter. These crude leachates, containing decomposition products of amygdalin together with any other soluble soil constituents, were used in these experiments to test the thesis that toxic substances can be produced in soil wherever peach root residues or amygdalin are acted on by certain soil microorganisms.

The direct method of Warburg (14) was used for measuring the oxygen uptake of the root tips in the various test filtrates. In each case 2.2 ml. of the filtrate was used along with the appropriate filter paper immersed in 0.2 ml. of 20% KOH placed in the center well in order to absorb the CO_2 produced. The Warburg reaction vessels were always prepared in pairs and three or four pairs of vessels each containing 2.2 ml. of the same test filtrate were used in the same run. The excised root tips were added to one of each pair of vessels and the other vessel served as control so that the oxygen uptake of the test solution alone could be determined. The difference in oxygen uptake between the two vessels was considered to be the respiration rate of the root tips in the given filtrate. Oxygen uptake of most test filtrates without the excised root tips rarely exceeded 3 μl. of O_2 per hour. Where the oxygen uptake of the test filtrates alone was too great the experiments were repeated by using more controls and, where necessary, the filtrates were passed through a Seitz filter before being used. The variations between respiration rates of root tips in the different pairs of vessels having the same test filtrates never exceeded 10% and usually were within 5% of each other.

To have an adequate number of replicates with each test filtrate only three different filtrates were tested at one time. Two of these were the suspected toxic filtrates containing water leachates of old peach orchard or other soils

together with varying amounts of microbial decomposition products of peach root residues or amygdalin. The third was a nontoxic filtrate composed of water leachates of similar soils without any of the above-mentioned decomposition products.

Two temperature ranges were used in the respiration studies. Most of the tests were run at 20° C. with 15 excised peach root tips in each reaction vessel, while some were also carried out at 30° C. with eight excised peach radicles in each vessel.

To avoid possible errors due to variations in respiration rates of root tip samples obtained on different days or to varying amounts of toxic substances present in the different filtrates, the experiments were so arranged that each separate run was a complete series in itself containing both the suspected toxic filtrates and the nontoxic filtrates. Root tips from the same sample were used for each new run so that the effects on respiration brought about by the toxic filtrates were compared in each case to the respiration rates of identical root tips in filtrates considered as being nontoxic, and the results from each of these runs were kept separate. To compare results of one day with those of another, the oxygen uptake of excised peach root tips in the nontoxic soil filtrate was considered to be the normal respiration rate of that particular root tip sample. Thus the inhibition of respiration in this nontoxic filtrate was assumed to be zero per cent. The oxygen uptake of root tips from the same sample but placed in the other test filtrates containing the suspected toxic substances were compared to the normal respiration rate and the decrease in oxygen uptake in the toxic filtrates was calculated as percentage of inhibition of respiration. In this way, by expressing the respiration rates of root tips in other filtrates in terms of percentage of inhibition of respiration of the normal, comparisons could be readily made of the influence of soil types and locations, varying amounts of peach or other root residues, chemically pure amygdalin, different incubation periods, autoclaving, and other factors on the respiration rates of excised root tips. Thus the less toxic filtrates producing inappreciable inhibiting effects on the respiration of peach root tips were found to have inhibition of respiration values ranging from 0% to 30% while the more toxic filtrates having intense inhibiting effects on respiration had values of from 40% to 90%.

The different types of filtrates used in this study are listed in Tables II and III. Their method of preparation was similar in all cases to those described earlier. Tests with each of these types of filtrates were repeated on at least 10 different occasions using in each case freshly prepared test filtrate and the results presented are summaries of the data obtained. Most of the soils used in these tests were from old peach orchards. The samples had a pH range of 4.7 to 5.6, so that most of the test filtrates were within this range. A pH of about 5.3 was most common. The filtrates which were within this range were used without being adjusted while the few beyond this range were adjusted to a pH of approximately 5.3. The effects of the various test filtrates on respiration of excised peach root tips are summarized in Fig. 1.

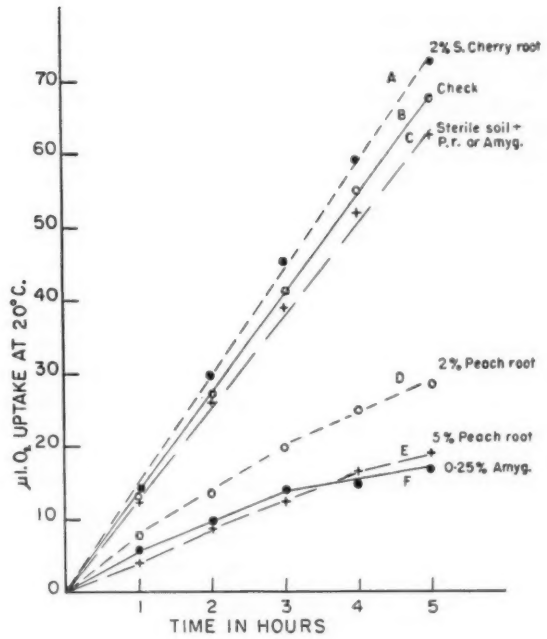

FIG. 1. Oxygen uptake of 15 excised peach root tips in soil–water leachates containing microbial decomposition products of: peach root residues (*D* and *E*; 2 and 5% indicate the amounts of the substances added, i.e. 2 and 5 gm. root residues to 100 gm. soil); amygdalin (*F*); sour cherry or other root residues (*A*); soil autoclaved before the addition of peach root residues or amygdalin (*C*); and soil-water leachates containing none of these substances (*B*). Corrections were made for the O_2 uptake of the leachates without the excised peach root tips. The soil (old peach site), incubation time (four days at 25° C.), preparation and extraction method was the same in all cases.

The respiration rate of the root tips in any of the test filtrates is given as the difference between the oxygen uptake of the test solution containing the excised peach root tips minus the oxygen uptake of the same test solution but without the root tips. Curves, *D*, *E*, and *F* show the respiration of 15 peach root tips in water leachates of old peach orchard soils to which 2% or 5% of peach root residue or 0.25% of amygdalin had previously been added. These toxic filtrates were obtained after incubation of three to four days at 25° C. at a time when very active microbial degradation of these substances was taking place as determined by the Na picrate paper test for CN. The normal respiration rate of root tips in water leachates of similar soils, but ones to which no peach root residue or amygdalin had been added, is shown by correlation line *B*. When sour cherry root residues were added to the soil the test for CN was negative and line *A* shows that very little or no effect on respiration of peach root tips was produced in leachates containing sour cherry root residues. When other root residues, such as tomato, tobacco, pepper, as

well as sour cherry roots, were added to the soil and water extracts made after the usual incubation period, no inhibition of respiration was obtained. The curves obtained in such leachates were essentially similar to those for sour cherry root residues, and show that during the microbial decomposition of these root residues no substances which inhibit the respiration of peach root tips are produced. In many instances when some of these root residues other than peach root residues were acted on by the soil microorganisms occurring in old peach orchard soils, substances were produced which stimulated the respiration of excised peach root tips. When the soil was first autoclaved and peach root residue or amygdalin were added, and the flasks incubated as above, a negative test for CN was obtained indicating that no breakdown of these substances had occurred. The water leachates which were then obtained produced very little or no inhibiting effect on the respiration of peach root tips as shown in line *C*. Similarly when peach root residue or amygdalin were added to compost or muck soils again no CN was detected, and the extracts obtained had a stimulating rather than an inhibiting effect on the respiration of peach root tips.

From the information in Fig. 1 it is readily apparent that substances which inhibit the respiration of peach root tips are produced only when peach root residue or amygdalin are acted on by certain soil microorganisms. If these organisms are eliminated or soil types used where these microorganisms do not occur in large numbers, such inhibiting substances are not produced. Similarly no inhibiting substances are produced when root residues other than peach are added to these soils.

As was mentioned earlier, since it is suspected that the amygdalin, which is a normal constituent of peach root bark, is mainly responsible for the toxic quality of peach roots, chemically pure amygdalin was also used in these studies to determine whether similar inhibitors are produced if the chemical alone is added to the soil. From the results presented in Fig. 1 and later, it is readily apparent that substances which inhibit the respiration of peach root tips are also produced when this chemical is acted on by soil microorganisms occurring in old peach orchard soils. The close similarity between curves *E* and *F* may also give some indication as to the approximate amygdalin content of the peach root residue used in these experiments. The curves show that filtrates from 5 gm. of peach root residue added to 100 gm. of soil and incubated for four days at 25° C. produced much the same inhibition as filtrates from 0.25 gm. of chemical amygdalin added to the same amount of identical soil and incubated for the same length of time. It appears, therefore, that a relatively large amount of amygdalin, as much as 50 mgm. per gram of root tissue, is present in Lovell peach roots and that as much as 5% of the dry weight of peach root bark may be in the form of this glycoside or its components. As shown by curve *D*, with filtrates obtained after 2% of peach root residue was added to the soil and incubated as above, the inhibition of respiration was a little less than with 5%. This depressing effect, however, was still severe producing up to 60% inhibition of respiration as compared to the check.

It was further noted that the toxic filtrates obtained were physiologically very active and produced an almost immediate inhibiting effect on the respiration of peach root tips and a measurable reduction in oxygen uptake even when the root tips remained in the toxic filtrates for less than half an hour. The inhibiting effect was also much more severe when respiration was measured at 30° C. At the higher temperature the respiration of peach root tips in the toxic filtrates was approximately 35% of the normal during the first two hours, and only 10% of the normal or 90% inhibition of respiration was obtained after the fourth hour. In many instances very little or no oxygen uptake took place or could be measured after the peach root tips had been in the toxic filtrates for four hours.

(*b*) *Effect of Toxic Filtrates on the Apical Meristem of Peach Roots*

It was noted that, when the excised peach root tips were placed in the toxic leachates, their meristematic regions darkened and finally turned brown, while those placed in the other nontoxic filtrates showed no darkening or other deleterious effects. This peculiar darkening of the meristematic region was first noticed when the root tips were taken out of the Warburg reaction vessels after their respiration rates had been determined. Only those root tips which were in filtrates containing decomposition products of peach root residue or amygdalin showed this darkening (Fig. 8). Those root tips in filtrates where decomposition of these substances did not occur, or where other root residues were used, were not affected and their meristematic regions remained white (Fig. 11). Closer examination showed that this peculiar darkening was confined only to the region of the apical meristem and no visible injurious effect or discoloration was observed either in the region of the root cap or the region above the apical meristem. Microscopic examination of crushed mounts showed the affected cells to be filled with disorganized masses of brown granular and amorphous material. The cells were apparently dead. Similar browning was also produced when whole peach seedlings were placed in the above-mentioned toxic filtrates and no visible effect was obtained with seedlings placed in the nontoxic types of leachates.

Since browning of the tissues is usually associated with the polyphenol-oxidase enzyme (5), the substances present in the toxic filtrates apparently must react with this enzyme. No experiments, however, have yet been performed to verify this assumption.

This injurious and highly specific effect of the toxic substances on meristematic cells may be very significant in relation to the growth of peach roots. If the meristematic cells are injured or killed the growth of the root will cease until other cells are stimulated to meristematic activity. Where these toxic substances occur in high concentrations they may be partly responsible for some of the witches'-broom type of growth often observed in peach roots growing in locations where the replant problem is found.

(*c*) *Irreversibility of the Injurious Effects of the Toxic Filtrates on Peach Root Tips*

On several occasions after the oxygen uptake of peach root tips in the various toxic and nontoxic test filtrates was obtained the root tips were

removed from the Warburg flasks, rinsed in distilled water, and allowed to remain in sterile water overnight. The roots from each flask were kept separate so that the determinations could be made as to whether or not the inhibition of respiration and darkening of the meristematic region produced in the toxic filtrates is reversed after the roots have been immersed in water for 12 hr. The following day each of the root tip samples previously taken out of the various test filtrates were placed in Warburg flasks containing water and their oxygen uptake was again measured. The oxygen uptake of the peach root tips which formerly had been in the toxic leachates was very low, amounting to less than 10 μl. of O_2 in five hours, whereas the respiration of peach root tips which previously had been in the nontoxic filtrates was only slightly reduced from around 70 to about 60 μl. of O_2 in five hours. Apparently there had been no recovery from the injurious effects produced by the toxic leachates. Furthermore the peach root tips in the nontoxic leachates appeared white and healthy after the tests whereas those which previously had been in the toxic filtrates had turned darker and continued to have the brown meristematic region described earlier. It appears, therefore, that the peach root tips had been permanently injured or killed after an exposure of five hours to the toxic leachates and that the inhibition is not reversed after the tips have been in water for 12 hr. No experiments have yet been carried out to determine the longest time that peach root tips can be exposed to the toxic leachates and still recover when returned to water.

Amount of Toxicity as Affected by Incubation Time, Soil Source, and Other Factors

Whereas highly inhibiting and lethal effects on the respiration of peach root tips were brought about by the most toxic filtrates, it was observed that with other toxic filtrates the inhibitions produced were not so great. The amount of inhibition obtained varied from 40% to 90%. This was not unexpected since it is known that even highly toxic organic compounds eventually disappear from the soil (4, 6). The rate of disappearance depends on the proportion of organisms capable of carrying out toxic degradation and on soil conditions favoring this activity.

The amount of inhibition obtained with the different test filtrates was greatly influenced by many factors including that of whether or not the soil came from an old peach orchard, the amount of peach root residue or amygdalin added to the soil, the length of incubation period after these substances were added, and autoclaving. Some of the effects that these factors have on the relative toxicity of the resulting leachates are summarized in Tables II and III, the results being obtained in the same manner as has already been described.

The results in Table II show the relative amount of inhibition obtained when 2% and 5% of peach root residue or 0.25% of amygdalin were added to old peach orchard soil and incubated for increasingly greater lengths of time before extracts were made. The somewhat variable and incomplete

TABLE II

THE INFLUENCE OF INCUBATION TIME, SOIL TYPE, AND OTHER FACTORS ON THE RELATIVE TOXICITY OF THE RESULTING SOIL LEACHATES AS DETERMINED BY THE INTENSITY OF THEIR INHIBITING EFFECTS ON THE RESPIRATION OF EXCISED PEACH ROOT TIPS

Incubation time (hr.) at 25° C. before extracts made	Substances added to old peach orchard soil (site area)							
	Peach root residue		0.25% Amygdalin		Peach soil autoclaved		Sour cherry root residue 2%	2% Peach root residue added to "compost soil"
	2%†	5%	Site area soil	Intersite area soil	Peach root residue 2%	Amygdalin 0.25%		
	Per cent total inhibition (or stimulation) of respiration in 5 hr. at 20° C.*							
60	48	—	51	—	—	—	—	—
70	60	—	55	—	8	10 stim.	8 stim.	—
100	40	78	72	—	6	15 stim.	—	18 stim.
150	—	68	82	7 stim.‡	3 stim.	7 stim.	15 stim.	25 stim.
200	—	59	73	19 stim.	—	—	2	—
300	—	—	74	14	—	12	—	—

NOTE: ** Per cent total inhibition of respiration in five hours of 15 excised peach root tips placed in the various soil–water leachates (containing varying amounts of the decomposition products of the substances indicated) was calculated in the usual manner using as check (or normal respiration rate) the respiration of identical root tip samples placed in soil–water leachates containing none of these products.*

† Amount of peach root residues, amygdalin, or other root residues, calculated as % by weight of the total soil contents of the flask, added to each flask.

‡ If total O_2 uptake was greater than that of the check it was calculated as per cent total stimulation of respiration.

results may be due to differences between the various soil samples taken at different times or to other factors yet undetermined. The most active inhibition was obtained after a four-to-six-day incubation period at 25° C. whereas after about two weeks' incubation the test for CN was very weak, indicating that either all the amygdalin had been decomposed or very little breakdown took place at the time. Leachates obtained at this time, however, still gave strong inhibition of respiration of peach root tips. Similar leachates obtained after three or more weeks' incubation at 25° C. behaved erratically, some of them still producing varying amounts of inhibition while others induced stimulation of respiration. These results were considered to be abnormal and were not included here because under laboratory conditions unnatural changes such as souring or contamination would probably have taken place after such long incubation periods. There is some evidence, however, that the inhibition of respiration obtained with filtrates from microbial degradation of 0.25% amygdalin or its equivalent in peach root residue after two to three weeks' incubation is not very great, usually less than 30%.

It is also evident from Table II that the likelihood of toxic substances being produced when amygdalin is added to a soil sample is greatly influenced by the presence or absence in the sample of a microbial flora capable of utilizing the glycoside. In many instances when amygdalin was added to soil obtained from the intersite area negative results of a CN test indicated that no decomposition had taken place. Consequently no inhibition was obtained and often the respiration of peach root tips was slightly stimulated in these filtrates. A slight inhibition was obtained only after 300 hr. incubation. By this time, however, some decomposition of amygdalin had occurred. Similar results were obtained with filtrates from autoclaved as well as from compost soils. In these soils too, very little or no amygdalin breakdown had taken place and again some stimulation of respiration was obtained with most of the filtrates.

When sour cherry root residue was added to the soil in place of peach root residue no toxic substances were produced and no significant effect on respiration was obtained with these filtrates. This was somewhat surprising since sour cherry roots are said to contain some amygdalin (2). However, negative results in the test for CN when sour cherry root residues were added to the soil indicated that very little or no amygdalin breakdown was taking place.

Likewise, toxic substances were not produced when 10% of tobacco or pepper root residues were added to identical soils and incubated for varying time intervals. The effects of such filtrates on respiration of peach root tips is shown in Table III. Again some stimulation of respiration was obtained with these filtrates. The respiration studies summarized in Table III were carried out at 30° C. on eight excised peach root radicles. The toxic effects at the higher temperature were more pronounced than at the lower temperatures. The inhibition of respiration was more severe and the peach root tips at the end of three hours were discolored with browning in the region of the apical meristem. No such discoloration, inhibition, or other deleterious effects on

TABLE III

COMPARISON BETWEEN THE INTENSITY OF THE INHIBITING EFFECTS ON THE RESPIRATION OF PEACH ROOT TIPS PRODUCED BY DECOMPOSITION PRODUCTS OF PEACH ROOT RESIDUES, AMYGDALIN, AND OTHER ROOT RESIDUES AS INFLUENCED BY INCUBATION TIME AND SOIL TYPE

Incubation time (hr.) at 25° C. before extracts made	Substances added to old peach orchard soil (site area)										
	Peach root residue		Peach soil autoclaved								5% Peach root residue added to muck soil
	1%	5%	Amygdalin 0.25%	Peach root residue 5%	Amygdalin 0.25%	Sour cherry root residue 2%	Tobacco root residue 10%	Pepper root residue 10%	Calcium cyanide	
	Per cent total inhibition (or stimulation) of respiration in 5 hr. at 30° C.										
40	63	66	—	10 stim.	—	—	27 stim.	36 stim.	40	—	
60	68	76	—	12	4 stim.	4 stim.	—	—	—	6	
70	—	84	47	5	12 stim.	—	28 stim.	—	—	3	
80	55	81	—	14	—	7	—	30 stim.	—	—	
90	43	90	65	3 stim.	5	—	10 stim.	—	29	7	
110	—	83	68	—	13	5	—	—	—	—	

NOTE: *The method used to compare the inhibiting or stimulating (stim.) effects on the respiration of peach root tips produced by the different leachates is identical to that in Table II, the only difference being that the respiration studies were carried out at 30° C. on eight excised peach root radicles.*

peach root tips were observed even at the higher temperatures in the nontoxic filtrates. When 5% of peach root residue was added to muck soil very little inhibition of respiration was obtained with the resulting filtrates as compared to very pronounced inhibition, up to 90%, obtained when the same amount of peach root residue was added to old peach orchard soil. When, however, calcium cyanide was added to the soil and water extracts were made 40 hr. and 90 hr. later, 40% inhibition was obtained after 40 hr. and only 29% after the 90 hr. period. The cyanide apparently escaped quite readily from the soil.

From the results summarized in Tables II and III it appears that soon after peach root residue or amygdalin are introduced into old peach orchard soils toxic substances which inhibit the respiration of peach root tips are produced as a result of microbial degradation of these substances. The peak in toxicity of water leachates obtained from these soils is reached in from four to six days' incubation at 25° C. This peak is followed by a period of gradual decline in toxicity. Within two to three weeks, under ideal conditions for microbial activity and soil aeration, the toxicity resulting from the addition to the soil of 0.25% of amygdalin or its equivalent in peach root residue will gradually disappear. No toxic substances are produced unless microbial degradation of amygdalin has taken place, and consequently no significant effects on respiration were obtained with filtrates from autoclaved soils or from soils where peaches had not been grown.

In a few experiments, activated charcoal added to some of the toxic filtrates reduced by about half the toxicity of such filtrates. On the other hand, boiling for 10 min. reduced the toxicity of the filtrates by only 15 to 20%.

Respiration of Peach Root Tips in 0.25% Solutions of Amygdalin and Emulsin

Additional evidence to show that only the breakdown products of amygdalin are toxic to peach roots is presented in Fig. 2. The similarity of correlation lines shows that 0.25% solutions of amygdalin have very little or no effect on respiration. Likewise no effect on respiration is produced in 0.25% solutions of emulsin, the enzyme which hydrolyzes the glycoside into its components. When, however, the two substances were added together and allowed to react overnight at room temperature, a positive test for cyanide and marked inhibition of respiration was obtained in the resulting solution. Root tips in this solution showed the characteristic discoloration and browning of the apical meristem region already described, an effect which was not observed in peach root tips placed in the separate solutions of these substances or in water.

Effect of Water–Benzaldehyde Dilutions on Respiration of Peach Root Tips

Since benzaldehyde may be produced during amygdalin breakdown, the effect of this compound on the respiration of peach root tips was also tested and the results are presented in Fig. 3. Although benzaldehyde is only slightly soluble in water, no difficulty was encountered in making the various dilutions, and since no crystals were observed after the benzaldehyde had stood overnight it was also assumed that if any benzoic acid had formed it was in

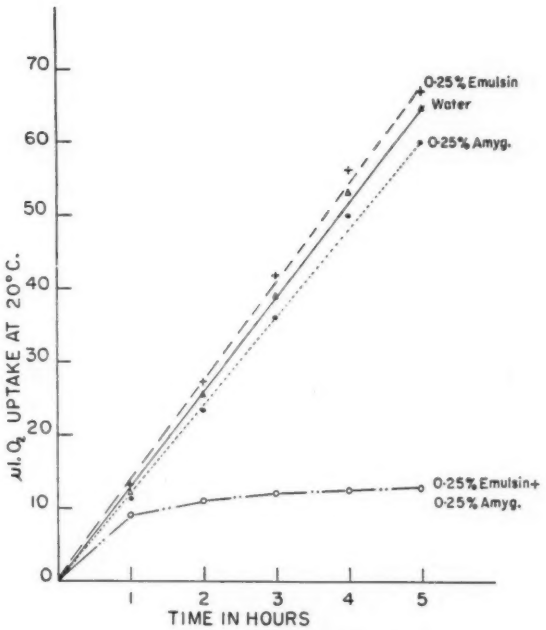

FIG. 2. Oxygen uptake of 15 excised peach root tips in water; water solutions containing either 0.25% amygdalin or 0.25% emulsin; and in solutions where the two substances (0.25% amygdalin + 0.25% emulsin) were combined and incubated 24 hr. at 25° C. before being used. The O_2 uptake of the solutions without the root tips was negligible and no corrections were necessary. Here, as in all these respiration studies, each Warburg vessel received 2.2 cc. of the test filtrate and 0.2 cc. 20% KOH in the center well.

solution. The results in Fig. 3 show that benzaldehyde also inhibits the respiration of peach root tips, the amount of inhibition depending on the amount of benzaldehyde in the solution. Some inhibition was obtained with dilutions as high as 1 : 5000. It was further noted that during the five-hour test period browning of the apical meristems was obtained with all dilutions up to 1 : 2000. No browning of apical meristems was obtained with peach root tips placed in the higher dilutions.

Other Toxic Effects

Although the main purpose of this investigation has been a study of the effect on the respiration of excised root tips of the substances formed when peach root residue or amygdalin are acted on by the microorganisms occurring in old peach orchard soils, a few experiments were carried out to see if other toxic effects were evident on whole peach seedlings under the influence of the same compounds. Most of these experiments were carried out in pots in the greenhouse; a few were also carried out in the field. The results obtained are described briefly.

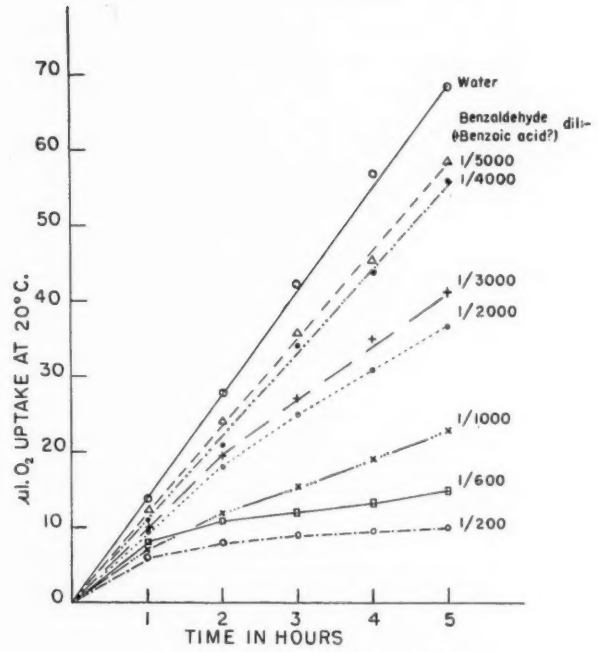

FIG. 3. Oxygen uptake of 15 excised peach root tips in water and in the various concentrations of benzaldehyde in water. The benzaldehyde-water dilutions were allowed to stand 24 hr. at room temperature before being used and some oxidation to benzoic acid may have occurred. The O_2 uptake of the different solutions without the root tips was negligible and no corrections were necessary.

A few 14-year-old peach trees were cut down, the stump being left in the ground in some cases while in others it was pulled out. Each location formerly occupied by a peach tree and the 10 surrounding feet constituted one plot. Thus each plot consisted of the area of the old site and continued out to the intersite. Some of these plots were fumigated with ethylene dibromide, others with methyl bromide, and some were not treated; and, in each, Lovell peach seedlings approximately five months old were planted one foot apart, starting in the area of the old site or close to the stump and continuing into the intersite 10 ft. away. Nematode population studies (11) as well as soil platings for microorganisms occurring in both areas before and after soil fumigation were conducted throughout the summer. Only a brief summary of the results will be given at this time. About three weeks after planting and continuing throughout the whole growing season the peach seedlings planted in the site area and up to 3 ft. from the site showed very poor growth. This stunting in the site area was present in both the fumigated and the non-fumigated plots. The seedlings planted 4 ft. beyond the site and into the

intersite grew very well. The site–intersite effect is shown in Fig. 4. The soil fumigants, ethylene dibromide and methyl bromide, gave some improvement of growth in the site area. The growth of peach seedlings in this area, however, was not nearly so good as that in the intersite area after fumigation. When the amount of growth of all the above-ground portions, including the main stem and side branches, was measured at the end of the growing season, the total growth measurements of seedlings planted in the site area varied from 40 to 90 cm. while total growth in the intersite area was 250 to 500 cm. It appears, therefore, that nematodes or other organisms which would have been greatly reduced by the soil fumigants were not responsible for all the stunting in the site area and that some of the stunting in this area was presumably due to toxic soil factors which were not eliminated by the fumigant used. As much as 40% of inhibition of respiration of excised peach root tips was obtained with water leachates of soil from this area. The large peach seedling on the left shown in Fig. 6 was obtained from the intersite area of the plot treated with methyl bromide whereas the stunted seedling on the right was obtained from the site area of the same plot.

In other tests carried out in pots in the greenhouse old peach orchard soil was used; 5 gm. of peach root residue for each 100 gm. of soil were added to some pots whereas no residue was added to others. Germinated peach seeds similar to those shown in Fig. 9 were put in both groups of pots and their growth was measured after nine days. The typical results obtained are shown in Fig. 10. The five seedlings on the left were grown for nine days in soil containing no peach root residue and the development of a long primary root and many secondary roots represents quite normal growth. The five seedlings on the right were grown for nine days in soil containing peach root residue and their root growth has been inhibited; the primary root is shorter and secondary roots are few and stunted. No root lesions were observed at this time, however, in either group of seedlings and the dark areas shown were due to soil particles adhering to the roots.

Other seedlings were grown in soil prepared in similar fashion and their roots examined 20 days after planting. These are shown in Fig. 7. Again the stunted seedlings on the left were grown in soil containing peach root residue while those on the right were in soil with no peach root residue. The root system in seedlings from soil containing peach root residues was greatly reduced and most of the growing tips were killed.

Into some of the leachates used in the respiration studies were placed 4-week-old peach seedlings. After three to seven days the seedlings in the toxic filtrates showed wilting symptoms and drying of the leaves starting from the bottom of the plant. The meristematic regions of the roots were also brown. Seedlings placed in the 1 : 2000 benzaldehyde dilution also showed this wilting, drying of the leaves, and browning of the meristematic regions of the roots. Seedlings placed in the nontoxic filtrates showed no injurious effects even after two weeks in the filtrates.

Isolation of Microorganisms Capable of Utilizing Peach Root Residue and Amygdalin

By means of the usual soil dilution method (8) a large number of microorganisms that are able to utilize the amygdalin in peach root residues were isolated from old peach orchard soils and from peach roots. The various soil samples, diluted 1 : 1000 to 1 : 100,000 with sterile water, were plated out on an agar medium containing as the nutrient source either peach root residue or amygdalin. The composition of peach root agar was: 50 gm. of ground peach roots, 100 cc. of soil extract, 900 cc. of water and 17 gm. of agar. These substances were added together, boiled until the agar melted, and filtered through several layers of cheesecloth and cotton. The resulting pH of the medium after autoclaving 15 min. at 15 lb. was about 6.3. The

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FIG. 4. A 14-year-old peach tree was removed and the plot was fumigated with methyl bromide. Five-month-old Lovell peach seedlings were then planted one foot apart starting at the old site (indicated by the remaining stump) and continuing into the intersite. Three weeks after planting and continuing throughout the whole growing season the growth of seedlings approximately 3 ft. or less from the old site was greatly retarded. Although the microorganisms and nematodes in this area were greatly reduced by the soil fumigant other factors detrimental to the growth of the peach seedlings appeared to be only slightly modified. Growth of seedlings 3 ft. or more from the site and in the intersite was, however, greatly improved.

FIG. 5. The uneven distribution of soil microorganisms that utilize peach root residue or amygdalin. The two Petri plates on the left contain peach root agar and 1 : 10,000 dilutions of soil obtained from the intersite area of a peach orchard; those on the right are from similar dilutions of soil samples obtained from the site area. Many more organisms developed in the Petri plates containing peach root agar and dilutions of site area soil than in the platings containing dilutions of soil from the intersite. Apparently a higher proportion of amygdalin-utilizing organisms are present in the site area.

FIG. 6. Differences in growth of peach seedlings planted in the site and intersite areas. The two seedlings were removed in the fall from the plot treated with methyl bromide, shown in Fig. 4, the stunted seedling on the right coming from the site area.

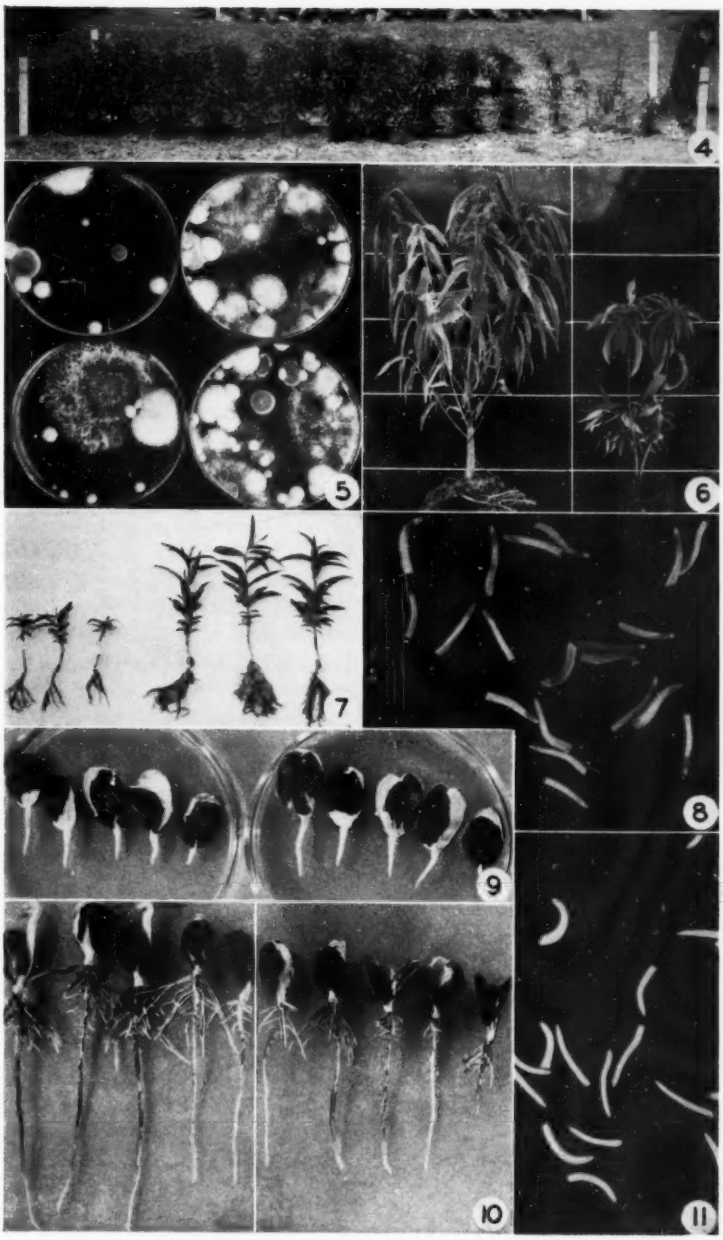
FIG. 7. Incorporating 5 gm. of peach root residues for each 100 gm. of soil to old peach orchard soil produced the stunting shown on the left 20 days after planting. The three peach seedlings on the right were planted in soil containing no peach root residues.

FIG. 8. This figure shows the brown, discolored apical meristem regions which were produced when the excised peach root tips were placed in soil–water leachates containing microbial decomposition products of peach root residues and amygdalin, or in solutions containing amygdalin hydrolyzed by the emulsin enzymes and in the various benzaldehyde-water dilutions.

FIG. 9. Examples of the germinating peach seeds, showing the sturdy radicle, which were used (Fig. 10) to demonstrate the depressing effect on root growth produced when peach root residues were incorporated into the soil before planting.

FIG. 10. Into soil containing 5 gm. of peach root residue for each 100 gm. of soil, were planted five germinating peach seeds shown above. Nine days after planting the seedlings were carefully pulled out and growth was measured. The five seedlings on the right were planted in soil containing the peach root residues. Their primary root is stunted and the few secondary roots were also stunted. The five seedlings on the left were planted in soil containing no peach root residues. The radicles were longer and many more secondary roots were present. No lesions were seen on the roots at this time and the darkened areas were due to adhering soil particles.

FIG. 11. The meristematic regions of excised peach root tips placed in soil-water leachates containing decomposition products of root residues other than peach or in soil–water leachates which did not contain amygdalin degradation products did not exhibit the injury and discoloration shown in Fig. 8; their meristematic regions appeared white and healthy.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amygdalin agar was prepared by adding 5 gm. of amygdalin to 100 cc. of soil extract, 900 cc. of water, and 17 gm. of agar. The resulting pH after autoclaving was about 7.2.

Fresh samples of soils from old peach orchards were obtained from the site and intersite areas and soil dilutions were plated out on these two media. The organisms which appeared and continued to grow were isolated for further study. The different species of organisms which were isolated on these two types of media or the relative ability of the different isolates to decompose amygdalin have not been determined. It should be mentioned however, that both fungi and bacteria were found. Fusarium species and a sterile white fungus appeared most commonly on these media along with a grayish-yellow, mucoid bacterial type. Very few actinomycetes were able to grow on such media.

When strips of filter paper dipped in the Na picrate solution were placed in the Petri dishes containing the various soil dilutions the paper turned red which indicated that cyanide was given off and the organisms were apparently utilizing the amygdalin.

A very interesting relationship was observed between the relative numbers of microorganisms which developed on the two types of media and the source of soil samples used in making the dilutions. Many more organisms appeared when various dilutions of site area soil were incorporated into the peach root residue or amygdalin agar media than when similar dilutions of intersite area soil were plated out on these media. This is shown in Fig. 5 where the two Petri plates on the left contain peach root agar and 1 : 10,000 dilutions of soil obtained from the intersite area, those on the right are from similar dilutions of soil samples taken from the site area. About five times as many colonies developed in the Petri plates when site area soil was used in the platings than when soil from the intersite was used. This uneven distribution of amygdalin-utilizing organisms may be related to the greater amounts of peach root residues occurring in old site area. Before the relative importance and significance of this uneven distribution of amygdalin-utilizing organisms can be determined, however, additional experimental evidence resulting from the use of other soil types and soils from other peach orchards will need to be obtained.

Discussion

Although it has been shown that substances which are highly toxic to peach roots are produced when peach root residues or chemically pure amygdalin are acted on by certain of the microorganisms occurring in old peach orchard soils, it is difficult to transpose results obtained in laboratory experiments to conditions in the field. From the data here presented, however, certain points of interest have been brought out and, although it would be unwise to generalize at this time, certain relationships have been noted and some cautious interpretations as to the possible significance of some of the results to the peach replant problem may be desirable.

It is further realized that the toxicity test used to demonstrate the deleterious effects of the soil–water leachates containing products from the breakdown of peach root residue may not be a true reflection of the toxic nature of these products because many major changes in respiration do not necessarily reflect equally important changes in the utilization of energy (1, 5). However, coupled with the rapid inhibiting effect on respiration are the browning and lethal effects on meristematic cells and these factors constitute strong supporting evidence for regarding the respiration-inhibiting substances in such leachates as being highly toxic to living peach roots.

The length of time these substances remain in the soil and their relative importance in inhibiting the growth of the peach replants are at present unknown. The answers are difficult to determine accurately and would vary with the many factors which may be different for each orchard. The relative importance of soil toxicity in the over-all peach replant problem can be estimated, however, by making use of some of the results obtained. Since the toxic leachates used in this study were produced by the interaction of peach root residues and microorganisms in old peach orchard soils—the two components also found in peach orchards having a replant problem—it is readily apparent that wherever peach roots or peach root residues occur, and as long as microbial decomposition of the amygdalin contained in these roots is taking place, soluble toxic substances highly detrimental to living peach roots are likely to be produced in these soils. The amount of these substances, and hence the degree of toxicity produced on the peach replant in the vicinity, will depend on the amount of old peach roots remaining in the soil after the old trees have been removed, as well as on their amygdalin content. They will also depend on the relative numbers of amygdalin-utilizing organisms in the area, and on the soil type and length of time that favorable conditions for microbial decomposition are maintained. In old peach orchard soils, especially in old site areas, there would be an abundance of roots left in the soil. It appears also that the Lovell variety, which is the most common peach rootstock used at present, contains a relatively large amount of amygdalin in its roots. As this amount may be as much as 5% of dry weight of the root, a relatively large quantity of amygdalin is likely to be left in old peach soils, especially in the old sites. Under most favorable conditions for microbial activity and aeration amygdalin is decomposed quite rapidly, hence under field conditions soil toxicity due to products of amygdalin decomposition may be expected to appear soon and to be quite high for some time after removal of the old peach trees. Peach replants planted during this period may thus be greatly stunted or even killed. The toxicity would gradually decline; the length of the toxic period and rate of decline would depend on the conditions mentioned. After all of the old roots had been completely decomposed most of the soil toxicity would also disappear. This disappearance would partly explain the observation frequently made that the toxicity of old peach orchard soils is often greatly diminished two to three years after removal of the old trees. This is to be expected since the toxicity of peach roots is mainly due

to their amygdalin content and the toxic effects of amygdalin are only produced during its breakdown. The HCN, one of the components of amygdalin breakdown, although highly toxic, diffuses out of the soil very rapidly so that the toxic effects due to cyanide occur only while it is being produced. The accumulation in the soil of benzaldehyde, the other toxic component of amygdalin, has not been determined. It is expected, however, that some of the benzaldehyde would be oxidized to benzoic acid. The toxicity of benzoic acid to peach roots is at present unknown. It is known, however, that other aromatic acids, as for example cinnamic acid, are toxic to plants (5, 6) while protocatechuic acid is toxic to fungi (5). It is suspected, therefore, that although benzoic acid is highly insoluble in water, it may also have toxic effects on peach roots. Whether or not other toxic substances are produced by the microorganisms utilizing amygdalin is also not known.

Besides the toxic substances from the microbial degradation of the amygdalin in the dead peach roots remaining in the soil after removal of the old peach trees, similar toxins could also be produced by living roots. This is due to the presence in peach root cells of amygdalin along with its specific splitting enzyme, emulsin (2). These two components are separated and thus no toxic substances are formed while the cells are intact. But as soon as the cells are disorganized the two substances are brought together and produce the toxins mentioned. These toxins could readily diffuse into the adjoining cells and give rise to the harmful effects described.

Although at present it is realized that there are many factors contributing to the over-all peach replant problem, because amygdalin is a normal constituent of peach roots it is evident that the toxic phase is intimately connected with the other phases. Thus any lesion-producing agencies such as nematodes, fungi, or insects, which could bring about the rupturing of the cells containing the two potentially toxic components would also aid in the production and release of the toxins and further intensify the root damage. From the work conducted at this laboratory (11) it is known that there are large numbers of parasitic nematodes in old peach orchard soils. Thus in addition to the damaging effects on the peach root system brought about by the nematodes themselves there is this second factor to consider, namely, the toxic effects produced by living peach roots when amygdalin and its splitting enzyme are combined as a result of nematode injury to the cells. These effects may also partly explain why other plant species planted in old peach orchard soils are not so seriously affected as peaches. Since their root cells do not contain toxin-producing components, injury to their roots through nematode feedings may not be so serious as similar injury to peach roots.

Acknowledgments

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CHRYSOMYXA IN NORTH AMERICA—ADDITIONS AND CORRECTIONS[1]

By D. B. O. Savile[2]

Abstract

Chrysomyxa ledi var. *glandulosi* Savile on *Ledum glandulosum*, *C. ledi* var. *groenlandici* Savile on *L. groenlandicum*, and *C. ledi* var. *vaccinii* Ziller on *Vaccinium parvifolium* are recognized as new varieties distinct from *C. ledi* var. *ledi* on *L. palustre*. Host and range extensions are given for several taxa, notably *C. ledi* var. *rhododendri* and *C. woronini*. The biology of the latter species is discussed.

Introduction

In the five years since the genus *Chrysomyxa* was monographed for North America (5), several new taxa have been recognized and many host and range extensions have been recorded. Dr. W. G. Ziller, Forest Biology Division, has discovered a new species and a new variety in British Columbia. Members of the Botany and Plant Pathology Division have added numerous records from Alaska and various parts of Canada. The acquisition of adequate material of *Chrysomyxa ledi* on *Ledum palustre* and *L. glandulosum* has made it clear that these plants and *L. groenlandicum* all support separate varieties of this rust. The new taxa, the chief range extensions, and revised keys are here presented.

Taxonomy and Distribution

Chrysomyxa ledicola (Peck) Lagerh., Tromsø Mus. Aarsh. 16 : 119. 1893.

This rust, already known from Newfoundland on *Ledum groenlandicum* and *Picea mariana*, was extremely heavy on the dominant *P. mariana* and the much scarcer *P. glauca* at St. Anthony, Nfld., in 1951. In August the scrub *P. mariana* and *Abies balsamea* clothing the hillsides, generally very similar in habit, were readily distinguishable at any distance by the bright yellow color of the former. Although *Chrysomyxa empetri* and *C. ledi* vars. were common, their aecial stages were difficult to obtain free from *C. ledicola*. This rust is clearly a major deterrent to the development of spruce in northern Newfoundland.

It may be noted that several specimens of *C. ledicola* from Japan (PUR and DAOM ex Herb. Hiratsuka), all somewhat scanty and overmature, yield spores slightly smaller and more finely warted than those of North America. The Japanese population has probably been isolated since preglacial times and may prove to be a distinct subspecies.

Additional records include: *Picea glauca*, Alaska, British Columbia, Newfoundland; *P. glauca* var. *albertiana*, British Columbia; *P. mariana*, Alaska; *P. sitchensis*, British Columbia.

[1] *Manuscript received May 24, 1955.*
Contribution No. 1462 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] *Senior Mycologist.*

CHRYSOMYXA EMPETRI (Pers.) Schroet. in Cohn, Krypt.-Fl. Schles. 3 : 372. 1887.

Uredinia were common on *Empetrum nigrum* at St. Anthony, Nfld., in 1951, but telia were scarce. Several collections thought to be of telia proved to be of immature uredinia. Collections of aecia on spruce later confirmed this scarcity, for, even in areas where *Empetrum* formed the dominant ground cover, *C. ledicola* was the dominant if not the sole needle rust. At Englee, some 50 miles south, *C. empetri* was light but prevalent on *Picea glauca* in one area far from *Ledum*. Infection of the current season's leaves of *Empetrum nigrum* was seen on 7 August at St. Anthony (Savile and Vaillancourt 2751). At this time aeciospores had been discharging for not more than a week; it is, therefore, clear that this infection was derived from the urediniospores produced in early summer. This is the only case of secondary infection in *Chrysomyxa* that I have seen in several years of field study.

It must be noted that one specimen previously cited as *C. empetri* on *Picea glauca* (DAOM 23436, Great Whale River, Que.) has recently proved to be mixed with *C. ledicola*. Holders of duplicates of this collection are asked so to annotate them.

Additional records include: *Empetrum nigrum*, Keew. (Chesterfield Inlet, *ca.* 300 miles beyond tree line), Labrador, Newfoundland. *Picea glauca*, Newfoundland.

CHRYSOMYXA PIPERIANA (Arth.) Sacc. & Trott., in Sacc. Syll. Fung. 21 : 716. 1912.

Uredinia of this species were collected in Manning Provincial Park, B.C., virtually at the northern limit of *Rhododendron californicum*, by Mr. W. Touzeau in 1952. Mr. J. A. Calder and I found it abundant in the same area in 1953, but could find no trace of aecia in the few adjacent spruce trees in either July or August. As previously indicated, the possible adaptation of this rust to a Mediterranean climate is worth investigation. In Manning Park it appears to be persisting in the uredinial state only. This species has been intercepted in a nursery shipment of *R. californicum* and may occur in plantings of the shrub in southern Vancouver Island, or about Vancouver.

CHRYSOMYXA LEDI (Alb. & Schw.) de Bary, Botan. Z. 37 : 809. 1879.

When my previous treatment of this complex was presented no satisfactory material of the European rust on *Ledum palustre* was available. One specimen examined bore a few overmature, collapsed urediniospores; the remainder, so labelled, consisted of germinating telia of *C. woronini*. Similarly, the few available specimens of the rust on the cordilleran *L. glandulosum* were scanty and overmature. I assumed erroneously, on the basis of approximate spore sizes, that these three host species harbored the same rust. Since that time we have received several Japanese specimens on *L. palustre* vars., and the only known collection on *L. palustre* var. *decumbens* (Muskox Lake, Back River, 64° 45′ N., 108° 10′ W., District of Mackenzie, J. G. Chillcott 135).

These specimens and one from Berlin, Germany (PUR F522), near the type locality, consistently differ from the rust on *L. groenlandicum* in having urediniospores with predominantly cylindrical rather than shallowly tabular warts.

The rust on *L. glandulosum* was collected repeatedly in southern British Columbia in 1953. This rust is very like that on *L. palustre*, but the urediniospore walls are consistently thicker. Each of these rusts must accordingly be treated as a separate variety.

Ledum groenlandicum was treated as a subspecies of *L. palustre* by Hultén (3), who minimized the distinctions between these plants in Alaska and considered that they intergraded almost completely. Although *L. groenlandicum* is sometimes small-leaved in Alaska, Yukon, and the northern half of British Columbia (including areas far from *L. palustre*), I cannot believe that these plants are properly to be regarded as conspecific. In the Hudson Bay region the two plants grow side by side without intergradation, and the morphological distinctions are supported by differences in flowering time and, to some extent, in habitat. That they are attacked by distinct varieties of *Chrysomyxa ledi* further supports the retention of *L. groenlandicum* as a full species.

Dr. Ziller's recent discovery of a member of this complex on *Vaccinium* brings the varieties to six. They are distinguished as follows.

CHRYSOMYXA LEDI (Alb. & Schw.) de Bary, Botan. Z. 37 : 809. 1879, var. LEDI.

Urediniospores 17–31 × 14–27 μ; wall, excluding warts, 0.5–1.0 μ; warts cylindric or rarely substellate, (0.6–)0.8–1.8(–2.2) μ high, 0.3–1.0(–1.4) μ wide or occasionally to 2.0 μ long, 0.6–2.0 μ spacing between centers (excluding elongate warts). Description based on one specimen from Germany (type area), five from Japan, and one from Muskox Lake, District of Mackenzie. The first-named specimen has the largest and the last the smallest spores of the series, but agreement is very close in all other characters and a longer series would probably wipe out the apparent distinction.

Hypophyllous on *Ledum palustre* L. vars., Europe, Siberia, Japan, northern Canada.

CHRYSOMYXA LEDI (Alb. & Schw.) de Bary var. **glandulosi** Savile var. nov.

Aeciosporae 16.5–22 × 14.5–20.5μ; parietes, verrucis exclusis, 1.0–1.6μ crass.; verrucae cylindraceae, 0.6–1.5μ alt., 0.3–0.8μ lat., 0.5–1.5μ a centro ad centrum.

In leaves of *Picea engelmanii* (Parry) Engelm., Manning Provincial Park, B.C. (Calder & Savile 11678, 10 August 1953, exclusively associated with 11677 on *Ledum*).

Urediniosporae 16–26.5(–28) × 14.5–21μ; parietes, verrucis exclusis, 1.0–1.6μ crass.; verrucae cylindraceae, 0.6–1.7μ alt., 0.3–1.0μ lat., 0.5–1.8μ a centro ad centrum.

Hypophyllous in *Ledum glandulosum* Nutt., Monashee Pass, B.C. (Calder & Savile 10109, 5 July 1953, (DAOM 45484) TYPE); Manning Park (C. & S. 11677), Hedley, Nickel Plate City, and McCulloch, B.C. (all in DAOM). Alpine, Mont. (Barth. N.A. Ured. 2625). Angora Lake, Calif. (Barth. N.A. Ured. 3327).

Isotype distribution: BPI, FH, MICH, PUR, TRT, IMI, O, S.

CHRYSOMYXA LEDI (Alb. & Schw.) de Bary var. **groenlandici** Savile var. nov.

Urediniosporae 19–34.5 × 12.5–20.5μ; parietes, verrucis inclusis, 1.0–2.0μ crass.; verrucae tesselatae, polygonatae, intervallis angustis, 0.5–2.5μ lat., vel ad ca. 7.0μ long.

Hypophyllous in *Ledum groenlandicum* Oeder, St. Anthony, Nfld. (Savile & Vaillancourt 2433, 21 July 1951 (DAOM 45442) TYPE). Common in all but the northernmost part of the host range. All specimens examined from British Columbia, Mackenzie, Alberta, Michigan, Ontario, Quebec, New Hampshire, and Newfoundland agree fully. Rel. Farl. 212 is certainly this variety, but is too overmature to serve adequately as the type. The packet of F. Columb. 354 in DAOM bears only germinating telia and is not positively identifiable.

Isotype distribution: BPI, MICH, PUR, TRT, IMI, S.

CHRYSOMYXA LEDI (Alb. & Schw.) de Bary var. CASSANDRAE (Peck & Clint.) Savile, Can. J. Research, C, 28 : 324. 1950.

Uredo cassandrae Peck & Clint. in Peck, Ann. Rept. N.Y. State Mus. 30 : 54. 1878.

Caeoma cassandrae Gobi, Scripta Botan. Horti Univ. Imp. Petrop. 1 : 169. 1886.

Chrysomyxa cassandrae Tranz., Trav. Soc. Nat. St. Petersb., Sect. Botan. 23 : 28. 1893.

The current interpretation of Art. 69 of the International Rules appears to be that if a specimen described as a *Uredo* also bears telia, unnoticed or unmentioned by the author, this specimen must be regarded as the type when the fungus is assigned to its appropriate genus; and the reassigning author must be regarded as making a new combination. If telia are lacking in the original specimen, the reassigning author is interpreted as making a new species based on his telial specimen. On the supposition that the latter was the case, Hylander, Jørstad, and Nannfeldt (4) state that my combination should have been based upon *C. cassandrea* Tranz. In his original description Peck cited specimens from Center, Sandlake, and Machias. The specimen from Sandlake does not appear to be in the Peck herbarium, but those from Center and Machias have been examined, and both bear germinating telia in addition to uredinia. The specimen from Center bears both stages in abundance and is the first one cited by Peck. It is accordingly hereby designated as the lectotype of *U. cassandrae* = *Chrysomyxa cassandrae* = *C. ledi* var. *cassandrae*. Under the current interpretation of Art. 69 my

combination is clearly valid. It may be noted that under a hand lens or the lowest power of a dissecting microscope the germinating telia look much like uredinia. Under magnification sufficient to show the urediniospores clearly they are readily distinguished.

Urediniospores 19–33.5 × 13.5–22μ; walls, excluding warts, 0.2–0.4 (–0.8)μ thick; warts ± cylindrical, 0.8–1.7(–2.2)μ high, 0.3–1.0(–1.5)μ wide or to 2.5μ long, 1.0–2.2(–2.5)μ spacing.

Hypophyllous in *Chamaedaphne calyculata* (L.) Moench, approximately throughout range.

Range extension: Alaska.

CHRYSOMYXA LEDI (Alb. & Schw.) de Bary var. **rhododendri** (de Bary) Savile comb. nov.

Uredo rhododendri DC., Fl. Fr. 5 : 86. 1815.

Chrysomyxa rhododendri de Bary, Botan. Z. 37 : 809. 1879.

Chrysomyxa ledi (Alb. & Schw.) de Bary var. *rhododendri* (DC.) Savile, Can. J. Research, C, 28 : 325. 1950, *nom. nud.*

De Candolle's material being unavailable, I must assume, with Hylander, Jørstad, and Nannfeldt (4), that it lacked telia. By application of the arguments given under the last variety my combination of 1950 must be considered invalid. It is accordingly remade in the hope that one such combination will withstand any future vagaries of the rules. In genera such as *Chrysomyxa*, in which the telia have little or no diagnostic value at either the generic or the specific level, such a situation is deplorable; but a completely satisfactory remedy is not easy to suggest.

Urediniospores (15–)17.5–29.5(–34) × 13–22μ; walls 0.3–0.6μ, excluding warts; warts ± cylindrical, 0.5–1.8μ high, 0.3–1.2μ wide or rarely to 1.6μ long, 0.7–2.2μ spacing.

Hypophyllous in leaves of *Rhododendron* spp.

Additional records include: *Rhododendron lapponicum* L., between Fort Nelson and Liard River, northern B.C., recorded by Anderson (1) as *C. roanensis;* Churchill, Man.; White Hills, near St. Anthony, Nfld. *Rhododendron* spp. (cult. vars.) Long Beach, Wash.

It will be seen that this rust is now recorded on *R. lapponicum* nearly from coast to coast. Observations at Churchill and St. Anthony suggest that it is relatively abundant after midsummer, but does not develop extensively until the bloom is passing. It is accordingly scarce in phanerogamic collections. The Washington records are from a nursery where many varieties were heavily infected. Although the source of this outbreak is uncertain, the inoculum was apparently introduced with imported nursery stock. When it is recalled that these rusts typically overwinter as dormant mycelium in the leaves, it becomes clear that the most vigilant plant quarantine inspection may fail to reveal them. Whether the outbreak can be contained depends in part on whether the native *R. californicum* proves resistant, as it well may. It is unlikely that the inoculum could have originated from *R. lapponicum*, for

plantings in coastal British Columbia, as far north as Prince Rupert, have apparently not been affected. The urediniospores in the Washington specimens are slightly smaller than those seen from Canada and Europe. Possibly they represent a race with slight morphological distinction; but the Washington outbreak probably stems from a very limited initial inoculum, and the rust may, in effect, be a clone that does not fully represent the natural variation of the parent population.

CHRYSOMYXA LEDI (Alb. & Schw.) de Bary var. **vaccinii** Ziller var. nov.

Uredinia hypophylla 0.1–0.5 mm. lat. vel usque 1.0 mm. long. Peridium corium simplex cellularum, cellulae peridii evascentes, polygonatae, 9–12μ diam. Urediniosporae (20–)22–32.5 × (13–)15–21.5μ; parietes, verrucis exclusis, 0.3–0.6(–0.8)μ crass.; verrucae cylindraceae vel leniter stellulatae, 0.5–1.8μ alt., 0.6–1.5μ lat., rariter usque 3.0μ long., 0.9–2.2μ a centro ad centrum. Telia inter urediniis sparsa, hypophylla, lutea, epidermide tecta, pulvinata, a rotundatis elongata ellipsoideaque, plerumque confluentia, 150–300μ long., 150–250μ lat., 80–130μ alt. Teliosporae catenulatae, ab irregulariter polygonatae oblongatae, 12–22 × 7–12μ, pariete leve, hyalino, 0.5–0.7μ crass. Basidia clavata, 25–30μ long., apice 6–8μ diam. Basidiosporae subglobosae, 8–11 × 6–8μ.

In the evergreen leaves of the previous year of *Vaccinium parvifolium* Smith. All specimens recorded are from British Columbia: Massett, 7 July 1952, DAVFP 7793; Massett, 8 July 1952, DAVFP 7776, DAOM 45774; Queen Charlotte City, 10 July 1952, DAVFP 9081; Tlell, 11 July 1952, DAVFP 9348; Qualicum Beach, 17 June 1955, DAVFP 9450, DAOM 46500 (TYPE); Qualicum Beach, 20 May 1953, DAVFP 9349. All collections by W. G. Ziller. DAVFP numbers are those of the Canada Department of Agriculture Forest Biology Laboratory, Victoria, B.C. Qualicum Beach is on the east coast of Vancouver Island; the remaining stations are from the Queen Charlotte Islands. Isotype material also in PUR.

Vaccinium parvifolium is a semi-evergreen species. It is perhaps more completely deciduous in the interior than in the outer coastal region of mild winters. If this is so it may account for the restriction of a rust dependent upon evergreen leaves to the latter area, but it is also true that restriction to a narrow coastal area in this general region is a relatively common pattern of distribution. Hultén (2) postulated the eastern part of the Queen Charlotte Islands as a pleistocene refuge. It is true that this area shows no indication of glaciation; and it is possible that, with the sea level substantially lowered during the glacial maxima, both this plain and the adjoining shallow sea to the east were exposed. On the other hand the heavy load of ice on the Queen Charlotte Islands mountains and, particularly, on the mainland coast ranges may have depressed this whole area below even the lowered sea level. It thus appears that we must rely on plant distribution patterns for the answer to this problem. In the Queen Charlotte Islands region most plants of adequately known distribution spread too far north or south to supply substantial

support for such a refuge. If the known distribution of this rust is nearly its full actual range, it would strongly suggest the occurrence of a Queen Charlotte refuge; but our mycological knowledge of the western cordillera does not yet warrant such an assumption.

There appears to be no previous record of a *Chrysomyxa* on *Vaccinium*, but, since the majority of species are fully deciduous, this lack is not very surprising. This rust is beyond question a typical *Chrysomyxa;* and, in view of its extremely close resemblance to the other members of the complex, the only practical course seems to be to treat it as a variety of *C. ledi* despite the fact that *Vaccinium* is not very closely related to the other hosts. Dr. Ziller reports that all specimens occurred close to *Picea sitchensis* and *Tsuga heterophylla;* and it is logical to expect that the former will prove to be an aecial host.

CHRYSOMYXA PIROLATA (Körnicke) Wint. in Rab., Krypt.-Fl. ed. 2, I, 1 : 250. 1882 (as *pirolatum*).

Aecidium ? *pyrolae* DC, Fl. Fr. 6 : 99. 1815. Not *Aecidium* (= *Puccini-astrum*) *pyrolae* Schulz, Prodr. Fl. Starg. 452. 1806.

Uredo pirolata Körnicke, Hedwigia, 16 : 28. 1877.

Chrysomyxa pyrolae (DC.) Rostr., Botan. Centralbl. 5 : 127. 1877, *nom. nud.*

Additional records include: *Picea glauca*, Norman Wells, Mack. *Pyrola chlorantha*, central British Columbia (*P. virens*, under which records on this plant were previously reported (5), is now recognized to be a synonym of *P. chlorantha*). *P. grandiflora*, Kenai Peninsula, Alaska. *P. picta*, Spuzzum, B.C. *P. secunda*, Kenai Peninsula, Alaska (and records in Anderson (1)).

CHRYSOMYXA MONESIS Ziller, Can. J. Botany, 32 : 435. 1954.

This recently recognized species is distinguished from *C. pirolata* by narrow fluted warts, instead of broad polygonal warts, on both aeciospores and urediniospores. It is essentially a coastal species, occurring from southern Alaska to Washington, but it penetrates inland to some extent. Ziller records it from Hope at the south end of the Fraser canyon. Calder, Savile, and Ferguson took it near the summit of Mt. Pope, Fort St. James, in central B.C. in 1954; but lowland specimens from the Nation River crossing, north of Fort St. James, and from Prince George, proved to be *C. pirolata*. Calder took it at Kenai Lake, Kenai Peninsula, Alaska in 1951.

Rusts, like their host plants, tend to spread much faster along the Pacific coast than inland, and the data do not make clear the location of the pleistocene refuge of *C. monesis*. If the refuge was in Beringia, it may be supposed that the rust occurs in Kamtchatka or further to the southwest, but the climatic tolerance of *Moneses* suggests that survival may have been in coastal Oregon or Washington. The unusual sculpturing of the aeciospore and urediniospore walls in *C. monesis* further emphasizes the close correlation between these spore forms in *Chrysomyxa*, which I have discussed elsewhere (5, 6).

Systemic rusts are frequently difficult to manipulate; and Dr. Ziller is to be congratulated for his prompt elucidation of this species.

CHRYSOMYXA CHIOGENIS Diet., Botan. Gaz. 19 : 303. 1894.

Chrysomyxa chiogenis occurs sparingly at St. Anthony, Nfld., and in central British Columbia, where *Chiogenes hispidula* occurs sporadically. The host occurs sparingly in Alberta, Saskatchewan, and Manitoba, and it now seems likely that the distribution of the rust is continuous across the continent. The study of abundant material in the field makes it clear that this rust is not systemic as I previously suggested (5). The effect is due to several successive leaves being in a susceptible stage of development during the period of aeciospore or urediniospore discharge. The practice of breaking up the trailing stems into short lengths for insertion in small packets heightens the illusion. The aecia must be quite rare in nature. Both in British Columbia and in Newfoundland I have collected sparingly rusted spruce needles in association with rusted *Chiogenes*, but in each case the aecia proved to be those of *C. ledicola*.

A trace infection preserved in TRT, from an inoculation on *Picea mariana* at Lake Temagami, Ont., in 1934 by W. R. Watson, yields aeciospores 21–27 × 16–21μ; wall *ca.* 0.5μ, excluding warts; warts 1.3–2.0μ high, 0.5–1.5μ wide or occasionally to 2.5μ long, 0.7–2.0μ spacing, often with irregular or fluted margins, columnar, and truncate rather than tapering as in *Chrysomyxa ledicola*. These dimensions are not conspicuously different from those of *C. ledi* vars. Although the spores probably are those of *C. chiogenis*, one would feel greater confidence had this specimen consisted of more than one needle. It is now apparent that this species is more closely allied to *C. ledi* than to *C. pirolata* as I previously supposed.

CHRYSOMYXA WORONINI Tranz., Centralbl. f. Bakt., II, 11 : 106. 1903.

Chrysomyxa woronini was relatively abundant in 1951 at St. Anthony, Nfld., where it was collected on *Ledum groenlandicum*, *Picea glauca*, and *P. mariana*. On one occasion no infected *Ledum* plants were found within 200 yd. of a light infection on *P. glauca;* but generally telial brooms could be found within a few feet of the aecia. The discovery of this rust on *L. groenlandicum* markedly changes its potential range. It now appears that its absence or scarcity in southern Canada may be due to the lack of sustained high humidity at infection periods. This species has recently been collected on *P. glauca* in Alaska (mile 187 Richardson Highway, T. J. M. Webster) and British Columbia (Fort St. John, T. M. Grant), and on *L. palustre* var. *decumbens* in Alaska (Kenai Peninsula, J. A. Calder, 3 collns.). It should be sought in coastal British Columbia, coastal Labrador, and the Cape Breton highlands of Nova Scotia.

A full understanding of this interesting rust requires observation of infected *Ledum* plants for two or more years in succession; but observations at St. Anthony threw some light on its biology. Although the mycelium is systemic

and perennial, occasional branches escape infection and, more significantly, only some of the leaves of infected shoots become invaded. As far as my observations extend, invasion of the first leaves that are produced in the spring is usually complete, but many or most of the later leaves escape infection although the mycelium must penetrate the twigs and enter the terminal buds. The telia almost completely destroy the lower epidermis of the infected leaves; and these leaves accordingly do not persist into the following season, as normal leaves do, but wither and fall late in their first season. It is thus clear that incomplete invasion of the host is an essential provision, without which the host would be killed. Other parasites may attack the uninvaded leaves, which seems partly to account for the confusion of this rust with *C. ledi* in Europe. Specimens harboring both rusts have been issued as *C. ledi* with the annotation that the uredinia are on the previous year's leaves and the telia on those of the current season. Actually the telia of *C. ledi* are always formed in the old leaves. The Alaska specimens cited above bear *C. ledicola* as well as *C. woronini*. One specimen from St. Anthony bore *C. woronini*, *C. ledicola*, and *C. ledi* var. *groenlandici*. Another shrub bore the first two rusts and one shoot uninvaded by *C. woronini* was invaded by *Exobasidium vaccinii*.

Revised Keys to North American Taxa

The recognition of one new species and three new varieties and a better understanding of *Chrysomyxa chiogenis* necessitate the preparation of new keys. In the key to peridermia no attempt has been made to separate the varieties of *C. ledi*. There is no certainty that the minor observed differences between these aecia are constant, and they are certainly too trivial to provide practical key characters. Dimensions may be approximate where they apply to a group of taxa.

Key to Peridermia

1. Scattered on leaves of *Picea*, or sparingly on cones.
   2. Spores usually lanceolate, *ca.* 70 × 20μ ..... *C. piperiana*
   2. Spores globose to ellipsoid
      3. Spores 27–47(–62) × 19–34μ
         4. Wall, excluding warts, 1.5–2.8μ thick; warts 1.5–3.0μ high ..... *C. ledicola*
         4. Wall, excluding warts, 0.3–1.3μ thick; warts 1.0–2.2μ high ..... *C. empetri*
         4. Wall, excluding warts, less than 0.5μ thick; spores to 27μ wide .... *C. roanensis*
      3. Spores 18–33 × 14–25μ
         5. Spore warts nearly uniformly distributed, cylindrical to slightly elongate.
            6. Warts generally entire ..... *C. ledi* vars.
            6. Warts often fluted on margin ..... *C. chiogenis*
         5. Unknown; warts probably cylindrical, entire, and lacking from part of spore ..... *C. ilicina*
1. More or less systemic in cones of *Picea;* warts often elongate.
   7. Warts crowded, entire, polygonal ..... *C. pirolata*
   7. Warts widely spaced, fluted, narrowly elongate ..... *C. monesis*
1. Systemic in stunted shoots and leaves of *Picea* ..... *C. woronini*

Key to Uredinia or Telia

1. Uredinia present, usually conspicuous
   2. Uredinia localized
      3. Urediniospores lanceolate ..... *C. piperiana*
      3. Urediniospores ellipsoid
         4. Urediniospores 25–45 × 20–34 μ
            5. Epiphyllous; urediniospores to 34 μ wide
               6. Urediniospore wall, excluding warts, 0.4–1.8 μ; warts mostly stellate to slightly irregular, 1.0–3.0 μ high, 0.4–2.0 μ wide ..... *C. ledicola*
               6. Urediniospore wall, excluding warts, 0.3–1.0 μ; warts cylindrical to slightly stellate, 0.7–2.2 μ high, 0.3–1.0 μ wide ..... *C. empetri*
            5. Hypophyllous; urediniospores to 26 μ wide ..... *C. roanensis*
         4. Urediniospores 16–34 × 13–22 μ
            7. Warts generally absent from part of spore; on Ilicaceae ..... *C. ilicina*
            7. Warts ± evenly distributed; on Ericaceae
               8. Warts 0.5–1.3 μ wide, often to 5 μ long, usually conspicuously fluted ..... *C. chiogenis*
               8. Warts 0.5–2.5 μ wide, often to 7 μ long, polygonal, entire ..... *C. ledi* var. *groenlandicae*
               8. Warts 0.3–1.5 μ wide or to 2.5 μ long, entire or slightly fluted
                  9. Spore walls 1.0–1.6 μ thick ..... *C. ledi* var. *glandulosi*
                  9. Spore walls 0.5–1.0 μ thick ..... *C. ledi* var. *ledi*
                  9. Spore walls 0.2–0.6 μ thick
                     10. Warts 0.6–1.5 μ wide, rarely to 3.0 μ long, entire to substellate ..... *C. ledi* var. *vaccinii*
                     10. Warts 0.3–1.0(–1.5) μ wide or to 2.5 μ long, entire ..... *C. ledi* var. *cassandrae*
                     10. Warts 0.3–1.2 μ wide or to 1.6 long, entire ..... *C. ledi* var. *rhododendri*
   2. Uredinia systemic
      11. Urediniospore warts crowded, entire, polygonal ..... *C. pirolata*
      11. Urediniospore warts widely spaced, fluted, narrowly elongate ..... *C. monesis*
1. Uredinia lacking
   12. Telia systemic, on *Ledum* ..... *C. woronini*
   12. Telia localized, on *Picea* ..... *C. weirii*
   12. Telia localized, on *Arctostaphylos* ..... *C. arctostaphyli*

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SPECIES OF SOOTY MOLDS FROM WESTERN NORTH AMERICA[1]

By Margaret E. Barr[2]

Abstract

A study of a capnodiaceous fungus occurring on western conifers resulted in the investigation of several new or little-known species. This paper includes an emended description and developmental study of *Limacinia alaskensis* Sacc. and Scalia, from *Alnus* and various conifers. The following new species, new combination, and new variety are also described from western hosts: *L. multiseptata* sp. nov., *Capnodium spongiosum* sp. nov., *L. moniliforma* (Fraser) comb. nov., and *L. moniliforma* var. *quinqueseptata* var. nov.

In the course of an investigation of some Pyrenomycetes of British Columbia the author examined specimens in the Herbarium of the Forest Biology Laboratory at Victoria. One collection on twigs of *Abies*, at first glance reminiscent of *Herpotrichia nigra*, could not be identified at that time. Later Mr. W. G. Ziller kindly forwarded further specimens on *Abies* and other conifers and from the material available a description was compiled which indicated that the fungus was a member of the Capnodiaceae. For comparative purposes specimens of sooty molds were obtained from the Herbaria of the Universities of Michigan and California, the Missouri Botanical Garden, and the State College of Washington. Among these collections were found three species belonging to the Capnodiaceae which were previously undescribed or which had not been reported from the Northern Hemisphere. These species are herein described and discussed, in addition to the original species mentioned. In indicating the herbaria in which the specimens are found the following designations are used: (DAOM) Mycological Herbarium, Division of Botany and Plant Pathology, Ottawa; (DAVFP) Herbarium of the Forest Biology Laboratory, Victoria, B.C.; (MO) Herbarium of the Missouri Botanical Garden; (UC) Herbarium of the University of California; and (WS) Herbarium of the State College of Washington.

The sooty molds present a confused picture, owing in part to the frequent presence in one collection of several conidial and ascomycetous entities, in part to incomplete or confusing descriptions of species previously named, and in part to divergent viewpoints of the various authors on the limitation of genera and species. Miss Fisher's (7) emendation of the diagnosis of the family Capnodiaceae and her conservative interpretation of the genera involved, which follows closely that of von Höhnel (14), is here adhered to. The genera of this family are distinguished on the basis of spore color and septation. The species here discussed fall into two genera: *Limacinia* with brown, transversely septate spores, and *Capnodium* with brown, muriformly septate spores.

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Contribution No. 1038 from the Department of Botany, University of Michigan, Ann Arbor, Michigan.

[2] *Graduate Student in the Department of Botany.*

These two genera are obviously closely related. The original generic lines have been enlarged to include numerous species which deviate in one or more characteristics. The extremes of the two genera are clearly distinct, thus the elongate ascostromata of characteristic species of *Capnodium* may be readily separated from the subglobose to ellipsoid ascostromata originally described for *Limacinia*. Ascostromatal shape is, however, extremely variable and is apparently dependent on the environment to a large extent (Fraser (9, 10), Fisher (7)), so that converging species are to be found which can only be separated on the basis of spore septation. This character may be thought to be an artificial means of separation, but until a thorough revision of the genera of this group of fungi is undertaken it should be retained.

In many collections of sooty molds a bewildering array of conidial forms is to be found. Pycnidia, spermogonia, synnemata, and free conidia formed on the hyphae have all been described for members of the family. Certain investigators have made cultural studies in an effort to determine the relationships of these various forms and their place in the life cycle (Neger (20), Fraser (8, 9)). Definite connections are difficult to make. However, Miss Fraser (9) found evidence of pycnidia connected with species of *Aithaloderma* and *Capnodium*. In this study of dried material no cultural experiments were possible. The conidial forms found occurring with the species here described are characterized briefly, in order to provide a record of their presence as components of the sooty mold colony and to facilitate further studies on the life cycles of these fungi.

Limacinia Neger

The genus *Limacinia* was proposed by Neger (in Johow (15)) to accommodate the species *L. fernandeziana*, found on the leaves and branches of various genera of trees and shrubs in the archipelago of Juan Fernandez, off the coast of Chile. The fungus was discussed earlier (Neger (19)) under the name *Antennaria scoriadea* Berk., at which time Neger suggested the formation of a new genus to include this characteristic form. In its original sense the genus included only the type species, which had dark, branching, moniliform hyphae frequently forming a large mass or subiculum; spherical or ellipsoidal perithecia, dark and glabrous, lacking a pore, and opening irregularly at the apex at maturity; oblong asci united at their bases, 8-spored, without paraphyses; spores colorless and two-celled when young and four-celled and grayish black at maturity, cylindrical or wedge-shaped. Spermogonia found attached to the hyphae were very small, pyriform, and contained numerous minute, hyaline cells. Conidia were described as fusoid–cylindrical and multiseptate, and were formed at the tips of elongate columns consisting of agglutinated hyphae. Neger considered his genus closely allied to *Perisporium*.

Von Höhnel (12) considered *Limacinia* a true "Capnodiee". He also studied *Antennaria scoriadea* Berk. (13), concluded that it belonged to the Capnodiaceae, and designated it as *Capnodium scoriadeum* (Berk.) v. Höhnel.

Neger's fungus, he said, differed from Berkeley's in having transversely septate rather than muriform spores; they were otherwise closely related. Von Höhnel (14) provided a key to the Capnodiaceae in which *Limacinia* Neger and *Perisporina* P. Henn. were recognized as brown, phragmosporous genera of the family. *Perisporina* was distinguished in this key by the cylindrical spores arranged in a fascicle in the ascus. Miss Fisher (7) considered that the generic distinction was neither adequate nor accurate and included *Perisporina* as a synonym of *Limacinia*.

Saccardo (22, 27) included *Limacinia* in his tribe Capnodieae of the Perisporiaceae. He characterized the genus as *Capnodium* with subglobose perithecia and oblong, sub-triseptate, hyaline to brown, rarely muriform spores. He included species with glabrous or appendaged perithecia in the genus, and did not remark on the presence or absence of an ostiole. A tabulation of species of *Limacinia* listed in the volumes of his Sylloge Fungorum (22, 23, 24, 25, 26), including only species having transversely septate, brown spores yielded the following: ascostromata astomous, glabrous—three species; ascostromata astomous, appendaged—one species; ascostromata ostiolate (at least with pore at maturity), glabrous—seven species; ascostromata ostiolate, appendaged—five species.

In 1910 Arnaud (2) discussed the genus *Limacinia* and placed in it species with globular perithecia, undifferentiated (i.e. exhyphopodiate) mycelium, and transversely septate spores. He included both hyaline and brown-spored species in his interpretation of the genus, which he subdivided on the basis of the presence or absence of appendages on the perithecia into *Eu-Limacinia* (glabrous) and *Morfea* (hairy or setose). Arnaud considered that an ostiole was probably present in all species but generally escaped notice. He placed this genus in the Sphaeriaceae, as he did with the other genera of the Capnodiaceae. In this paper Arnaud discussed and figured, or merely repeated descriptions of, 10 species in *Eu-Limacinia* and nine species in *Morfea*.

Theissen and Sydow (30) provided a key to the genera of the family Capnodiaceae and erected several new genera to accommodate species which they considered as diverging from the recognized genera. They misinterpreted *Limacinia*, considering it to have hyaline spores, which, as was previously shown, is not true of the type species and therefore not of the genus. Based on this misconception was the genus *Phragmocapnias* Theiss. and Syd. (30) which was erected to include species with brown spores, but similar in other respects to their interpretation of *Limacinia*. Another genus, *Setella*, was erected for a species with brown phragmospores whose perithecium was ornamented with one, rarely two, spines at the apex.

Spegazzini (in Saccardo (26)) proposed a key to the Capnodiaceae also. He too regarded *Limacinia* as hyaline-spored and erected numerous genera on what appear to be rather slight and variable characters. Later authors (Fraser (10), Miller and Bonar (18)) have followed Theissen and Sydow's interpretation of *Limacinia* and have described species with hyaline spores under this name. Miss Fisher (7), excluding many of the genera created by

Theissen and Sydow and Spegazzini, retained *Limacinia* for species with brown, transversely septate spores and *Scorias* for similar species with hyaline spores.

One other genus which should be considered in this discussion is *Aithalomyces* Woronichin (31). It was erected to include two sooty mold species from Russian Lapland and Siberia. According to Woronichin, the genus was distinct from *Limacinia* (in either von Höhnel's or Theissen and Sydow's sense) and from *Phragmocapnias* by the complete lack of an ostiole and by the "primitiven Anhängseln", i.e. mycelial and pendent rather than spiny and erect. It is possible that the species included in this genus belong in *Limacinia*; however, as no specimens were available for examination and comparison, they are not at present transferred to that genus.

It has been decided to retain the species here described in the genus *Limacinia* in the broad sense. The discrepancies found in descriptions of species in the literature make it appear that astomous or ostiolate conditions of "perithecia" are extremely variable and are not to be considered of generic significance. It has been observed in *L. alaskensis*, although no pore is visible in the apex when whole mounts are examined, that there is a distinctly differentiated area present in longitudinal section (Fig. 11). Our definition and interpretation of an ostiole is in itself extremely variable. Miller (17) discusses the various interpretations and the diagnostic prominence given to this term and limits the ostiolum to ". . . the canal passing through the papilla, or neck of the perithecium, and terminating in a pore. It is lined with minute periphyses, which are outgrowths of basal cells in the neck wall and have free ends. The pore is formed by the pulling apart of wall tissues at this point due to unequal growth, and the canal is formed by the upward growth of the wall. It is never formed lysigenously as in the Dothideales." By this use of the term the opening found at the apex of ascostromata of *L. alaskensis* and *L. multiseptata* would not constitute an ostiole. It is here referred to as a lysigenous pore.

The presence or absence of appendages on the ascostromata appears to be variable even within the species. The species of *Limacinia* discussed in this paper are appendaged, in the majority of cases, but occasional fruiting bodies were found in which the appendages were sparse or even lacking.

The genus is dothideaceous in nature (Luttrell (16)), according to the studies of Fraser (9), Fisher (7), and the author (see *L. alaskensis*). The so-called perithecium is here referred to as an ascostroma, in accordance with the terminology used by Miller (17), Luttrell (16), and others.

The genus *Limacinia*, then, is extremely variable. Its main characteristics are: presence of dark, entirely superficial hyphae which form an effuse or massive subiculum; ascostromata opening by a lysigenous pore or completely enclosed; appendages mycelial and pendent or stiff and erect, or lacking; asci (according to the species examined and to species figured in the literature) bitunicate, oblong to obclavate and saccate, aparaphysate; spores brown, ellipsoid, oblong, fusoid, or elongate-cylindrical, transversely two- to many-septate.

Limacinia alaskensis Sacc. and Scalia, Emend. (Figs. 1–17)

Limacinia ? alaskensis Sacc. and Scalia, Cryptogamic Botany, Harriman Alaska Series, 5 : 34, Pl. 4, Fig. 1. 1910.

Subiculum forming thin, dull or glistening black covering over twigs and leaves, entirely superficial, often several centimeters in length; composed of branched and anastomosing hyphae whose cells are longer than broad, slightly constricted at the septa, brown or grayish-brown, thick and smooth or slightly rough walled, containing one or more guttules, 9.5–25.5μ in length by 9.5–17.5μ in width. Ascostromata superficial or partially immersed in the subiculum, black, globose (104–192μ in diameter), conical (112–208μ in height by 104–200μ in width), or depressed-globose (104–112μ in height by 112–120μ in width); wall thin, composed of two layers of polygonal brown cells, the outer dark and heavy walled, without ostiolar differentiation, opening by a lysigenous pore at maturity; appendages hyphal, on the sides and toward the base of the ascostroma, anastomosing, occasionally branching, not or slightly constricted at the septa, 6.5–9.5μ in diameter, reaching a length of 64–264μ. Asci in a fascicle, oblong-ellipsoid, saccate below the middle, sessile or narrowed abruptly into a short, knob-like stalk, bitunicate, wall thickened toward the rounded apex, 8-spored, aparaphysate, 48–112 × 15–36μ. Spores biseriate when young, becoming irregularly crowded in the broad portion of the ascus, ellipsoid, ends broadly rounded, 3-septate, not or slightly constricted at the septa, yellow brown to dark brown, straight or slightly curved, containing one or more guttules in each cell, 20–37 × 8–13μ.

Habitat on branches of *Alnus* sp., and on twigs and leaves of *Abies amabilis*, *A. grandis*, *Abies* sp., *Pseudotsuga taxifolia*, and *Thuja plicata* in Alaska and Washington, United States of America, and in British Columbia, Canada.

Specimens examined: ALASKA, U.S.A.: On *Alnus* sp.—Glacier Bay, Muir Glacier, June 11, 1899, Trelease 712, COTYPE, MO 15343.

BRITISH COLUMBIA, Canada: On *Abies amabilis*—Cowichan Lake, V.I., Sept. 22, 1943, R. E. Foster, DAVFP 2491. Kitimat, June 14, 1954, A. C. Molnar, DAVFP 9239. Forbidden Plateau, V.I., June 23, 1954, C. Madhosingh, DAVFP 9222. On *A. grandis*—Cowichan Lake, V.I., June 12, 1948, W. G. Ziller, DAVFP 3209. On *Pseudotsuga taxifolia*—Cowichan Lake, V.I., Sept. 10, 1943, R. E. Foster, DAVFP 8332. On *Thuja plicata*—Kaslo, Aug. 8, 1948, A. W. Porter, DAVFP 8333. Shuswap Lake, May 21, 1954, B. G. Collins, DAVFP 9202.

WASHINGTON, U.S.A.: On *Abies amabilis*—near Adelaide Lake, Mt. Rainier Natl. Park, Sept. 15, 1935, H. E. Bailey, UC 620741, as *Phaeosaccardinula dematia* Miller and Bonar. On *Abies* sp.—Mt. Rose, Mason Co., Nov. 1949, C. J. Gould, WS 21430, type of *Helminthosporium abietis* W. B. Cooke and Shaw.

Through the kindness of Dr. C. W. Dodge, the cotype specimen of *Limacinia ? alaskensis* Sacc. and Scalia was obtained for study. Unfortunately the ascostromata yielded only immature asci, as the authors had noted (28) in their description. The hyphae which form the subiculum of this fungus are

entirely similar (cf. Figs. 3 and 3A) to those of the fungus on conifers from British Columbia and Washington. The young and older ascostromata also have the same method of formation and general appearance (cf. Figs. 8, 9, and 10). No conidia were found on this specimen; Saccardo and Scalia (28) described as conidia certain oblong–fusoid, brown, 3-septate, scarcely constricted, 28–30 × 10μ spores, which both from the description and from the figures appear to be identical with the ascospores of the fungus on conifers, and were probably discharged spores caught on the hyphae. There was no mention of any attachment of "conidia" to the hyphae, which strengthens this assumption. It is concluded from the examination of cotype material and from the description and figures previously referred to that *Limacinia ? alaskensis* Sacc. and Scalia is truly a species of *Limacinia* and that the fungus on conifers is identical with it.

An earlier name which may well be applicable to this fungus is that of *Capnodium* (*Limacinia*) *resinae* Sacc. and Bres. (3). The rather brief description includes both perithecial and conidial stages, the latter named *Helminthosporium resinae* Bres. The description of this fungus from northern Italy makes it apparent that it is closely related to the fungus on conifers and *Alnus*. Dr. S. J. Hughes, in a letter to the writer, remarked on this possibility and also stated that the conidial stage probably belongs in the genus *Hyphosoma* Sydow (29). No mention is made, however, of appendages on the perithecia, and Arnaud (2) includes this species with those placed in the glabrous subgenus *Eu-Limacinia*.

Two fungi described from England, *Capnodium juniperi* Phillips and Plowright (21) and *Asterina juniperina* Cooke (4), enter into the question of the identity of *Limacinia alaskensis*. Both species have perithecia which are seated on a black antennaroid subiculum and 3-septate, brown spores. The descriptions are very brief and leave doubt as to the exact nature of the fungi described. *Capnodium juniperi* is said to have globose-cylindrical perithecia contracted at the bases and spores with pointed ends. These characters would separate it from *Limacinia alaskensis*. The second fungus, *Asterina juniperina*, was transferred to *Phragmocapnias* by Theissen and Sydow (30), apparently without examination of the type specimen. Dr. R. W. G. Dennis,

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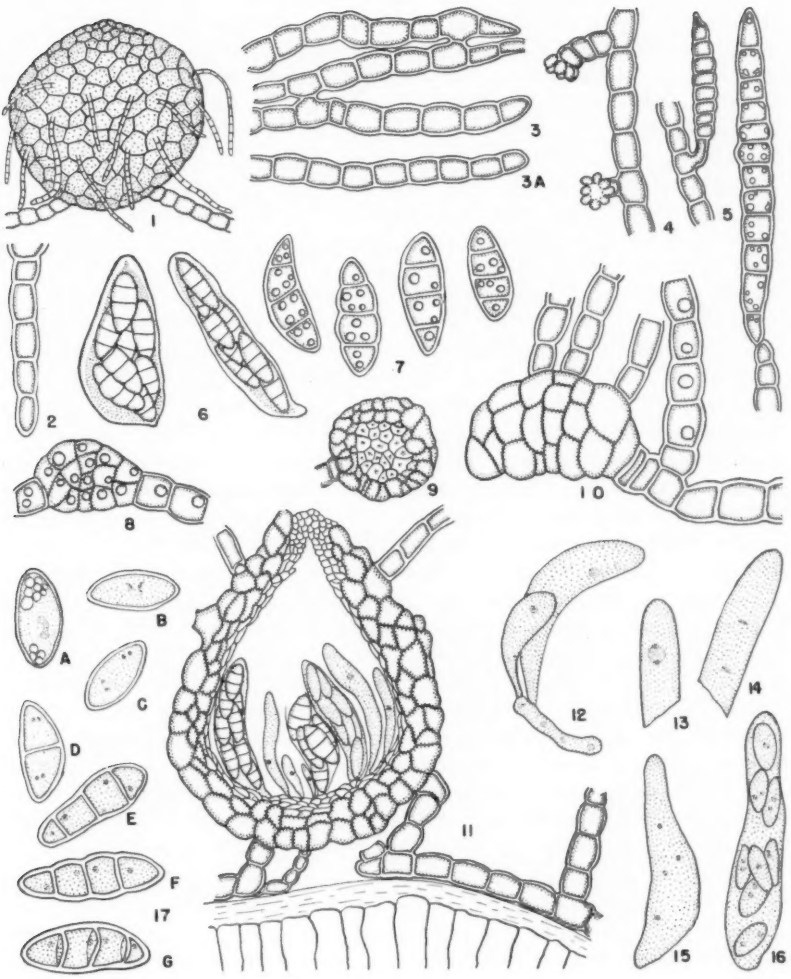
FIGS. 1 to 17. *Limacinia alaskensis*.

FIG. 1. Habit of ascostroma. FIG. 2. Hyphal appendage of ascostroma. FIG. 3. Anastomosing hyphae of subiculum. FIG. 3A. Hyphal branch from cotype material. FIG. 4. Phialides on short hyphal branches. FIG. 5. Two conidia showing attachment to hyphae. FIG. 6. Two asci. FIG. 7. Ascospores. FIG. 8. Immature ascostroma. FIG. 9. Section through immature ascostroma. FIG. 10. Immature ascostroma from cotype material. FIG. 11. Longitudinal section of mature ascostroma superficial on *Thuja*. FIG. 12. Two asci each containing a fusion nucleus and arising from a multinucleate cell. FIG. 13. Stage of first meiotic division. FIG. 14. Binucleate ascus. FIG. 15. Quadrinucleate ascus. FIG. 16. Delimitation of spore initials. FIG. 17. A-G. Stages in nuclear division and septum formation in spores. A. Uninucleate spore initial. B. Binucleate spore shortly after division. C. Second nuclear division. D. First transverse septum formed. E. and F. Four uninucleate cells of spores. G. Mature spore with wall cut away showing a single nucleus in each cell. FIG. 1. ×165. FIGS. 2–6, 8–11. ×420. FIG. 7. ×630. FIGS. 12–17. ×950.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Director of the Herbarium at Kew, has kindly examined the type specimen of *Asterina juniperina* and has compared it with material of the fungus on conifers. He is of the opinion that the two species are distinct.

Conidia were found in all but one of the collections on conifers listed previously. They arise from branches of the hyphae, either from the tip of a branch or from a side cell abstricted from an interior cell of the hypha and float free in water mounts. The conidia are long fusiform, narrowed toward the ends, light grayish-brown to darker brown, straight or slightly curved, 7- to 14-septate, not or slightly constricted at the septa, often with several guttules in each cell, and measure 56–152 × 6.5–16μ (Fig. 5). Dr. S. J. Hughes (*in litt.*) states that these conidia belong to the genus *Hyphosoma* and that they are identical with those described by W. B. Cooke and Shaw (6) as *Helminthosporium abietis*. He is of the opinion that it is preferable to treat the two forms as distinct taxa, even with the strong circumstantial evidence of their relationship, until definite proof of genetic identity is obtained. This viewpoint is concurred with in the present paper, but it is well to point out that both conidia and perithecia are present in the specimen on *Abies* sp., which is the type specimen of Cooke and Shaw's *Helminthosporium abietis*.

In addition to the conidia, short branches of the hyphae were frequently found which bear at the truncate tips a cluster of six to eight pale brown, globose to ovate cells (Fig. 4). Dr. Hughes suggested (*in litt.*) that these may be phialides. Miss Fraser (10) described and figured similar structures for *Capnodium mucronatum* Mont. She described the formation of small, hyaline conidia which were apparently budded off from the inner walls of the ring of apical cells. No such conidia were observed in the material examined.

In order to determine the morphology of the ascostroma of *Limacinia alaskensis* small portions of twigs of *Thuja* were cut, embedded in paraffin following passage through a series of tertiary butyl alcohols, and sectioned at 10μ. The sections were stained in Heidenhain's iron hematoxylin. Many of the slides thus prepared showed sections through immature and mature ascostromata. Material obtained in this manner indicated that the mycelium did not penetrate the host cells but was closely appressed to the thick cuticle of leaves (Fig. 11) and to the bark of twigs. No evidence of the presence of haustoria was found. The mycelium completely enveloped the twigs in many instances and surrounded the closely appressed scale-like leaves.

The earliest indications of ascostromata found were small knots of brown, thick-walled cells, 20–30μ in diameter (Fig. 8), which when cut through showed one to two outer layers of thick-walled cells surrounding a solid interior mass of thin-walled, hyaline cells containing a single nucleus and deeply stained contents (Fig. 9). These knots are borne on branches of the hyphae and are formed by division of a single cell of the hypha, which may be either apical or intercalary in position, as was also evidenced by water mounts of the fungus. In later stages the wall is composed of two layers of brown, thick-walled cells, especially dark and heavy externally. From certain of the surface cells, irregularly scattered over the ascostroma, hyphal strands arise

at an early stage as protrusions of the cell (Fig. 10). These become several cells in length, brown, and heavy-walled, and form a scant tomentum which mingles with the hyphae of the subiculum (Figs. 1, 2). Within the wall of the ascostroma are one to three compressed layers of hyaline, narrow, elongated cells. At the apex these small, thin-walled, closely agglutinated cells form a wedge-shaped mass protruding toward the surface. At maturity this group of cells is ruptured by the expanding asci and leaves an irregular pore. The cells at the base form a broad, slightly convex cushion.

The asci arise from deeply staining multinucleate cells (Fig. 12), "ascogenous cells", in the basal cushion, and push upward through the disintegrating cells which previously filled the locule. Immature asci are oblong and contain a single large fusion nucleus, 1-2μ in diameter (Fig. 12), surrounded by heavily-staining granular contents. As the asci enlarge, pushing up nearly to the apex of the locule, nuclear division results in the formation of eight nuclei (Figs. 13–16). Delicate walls become visible, cutting out oval regions of cytoplasm around each nucleus, and at the same time a second nuclear division occurs (Fig. 16). The nuclei divide again within the spore thus delimited (Fig. 17, A–C); following the formation of four nuclei three transverse septa are laid down, the central septum being first evident (Fig. 17, D–F). The spore wall darkens and becomes thickened at an early stage and thereafter it is difficult to see the nuclei within it (Fig. 7). However, one mature spore was observed with the wall cut away along the upper surface, showing a single nucleus in each cell (Fig. 17 G). The asci at maturity are broadly ellipsoidal or oblong with the lower portion inflated and sessile or narrowed into a short knoblike stalk (Fig. 6). They are bitunicate and have the apical portion of the wall greatly thickened. At maturity no trace of interthecial tissue was seen. The cells which had previously filled the locule had disappeared completely, either disintegrating or being forced to the sides of the locule to form the compressed layers of inner tissue of the wall.

The method of formation of the locule of the ascostroma here described has been observed in other members of the Dothideales. Fraser (9) records a similar method of development in species of *Aithaloderma* and *Capnodium*. Arnaud's ((1) Pl. I, Figs. 1 and 5) illustrations of sections through perithecia of *Pleosphaeria citri* (*Limacinia citri* (Br. and Pass.) Sacc.) are very similar to those found in *Limacinia alaskensis*.

Limacinia moniliforma (Fraser) comb. nov. var. ***moniliforma*** (Figs. 18–22)

Capnodium moniliforme Fraser, Proc. Linnean Soc. N. S. Wales, 60 : 168, Figs. 44–52. 1935.

Subiculum superficial, dull black, massive, composed of much branched hyphae, deeply constricted at the septa, thick and rough or smooth walled, cells globose to flattened globose, 40μ in diameter in the older hyphae, mostly 11–20μ in length by 10–27μ in width. Ascostromata superficial, ovoid to globose, 150–205μ in height by 95–136μ in diameter, thin walled, of one to

two layers of polygonal brown cells, without a differentiated ostiole, the apex opening by a pore at maturity and then appearing lacerate; appendages spiny, clustered around the apex, remainder of the wall glabrous to the base which is clothed with hyphae similar to those of the subiculum, apical appendages short, stiff, erect, simple or occasionally branched, rough walled, constricted at the septa, yellow brown, 8–13μ in diameter at the base, 20–45μ in length. Asci in a fascicle, oblong, ventricose below the middle, sessile or abruptly narrowed into a short, thick stalk, bitunicate, wall thickened toward the rounded apex, 8-spored, aparaphysate, 64–112 × 15–27μ. Spores biseriate when young, at maturity irregularly crowded in the broad portion of the ascus, ellipsoid–fusoid, often inequilateral or slightly curved, ends broadly rounded, thick and smooth walled, yellowish to dark brown, 3-septate, not constricted at the septa, with several small guttules in each cell, 18–38.5 × 9–13μ.

Habitat on branches of *Arctostaphylos sensitiva* and *Arctostaphylos* sp., California, U.S.A.

Specimens examined: CALIFORNIA, U.S.A.: On *Arctostaphylos sensitiva*— Mt. Tamalpais, Marin Co., March, 1933, V. P. Mentzer, California Fungi 301, UC 498813, as *Aithalomyces rhododendri* Woronichin. On *Arctostaphylos* sp.— Fire trail up Mt. Tamalpais, Marin Co., March 31, 1926, H. E. Parks, 3065, two specimens, UC 276003, 294722, as *Aithalomyces rhododendri* Woronichin.

The specimens cited above were determined by V. Mentzer Miller (née Mentzer) (18) as *Aithalomyces rhododendri* Woronichin, on the basis of Woronichin's (31) description of that species. Woronichin described the perithecia of his fungus as globose, somewhat depressed, with appendages arising from the middle and lower half of the wall, mycelial and anastomosing, whereas in the California fungus the perithecia are ovoid to globlose, with spiny appendages arising in a tuft at the apex. Furthermore, these appendages are short and consist of globlose cells constricted at the septa, while on the contrary those of *Aithalomyces rhododendri* are said to be long and to consist of cylindrical cells. It is the writer's opinion that these fungi cannot be considered identical.

The description and figures of *Capnodium moniliforme* Fraser (10) agree well with this fungus. Although the ascostromata of the California fungus are slightly larger than is given for *C. moniliforme*, they are similar in shape and both bear an upright apical tuft of appendages (Figs. 18, 19). The asci and spores (Figs. 21, 22) of the fungus on *Arctostaphylos* are somewhat larger than the sizes (asci 65 × 15μ, spores 18–31.5 × 9–11μ) given for *C. moniliforme*, but their lower limits do include these sizes. It is considered that the California fungus is merely a large form of *Capnodium moniliforme* Fraser.

As understood here the genus *Capnodium* includes species of sooty molds with muriform spores. Inasmuch as Miss Fraser did not describe nor figure any longitudinal septum in the spores of her fungus and as the author has found none in the California material examined, the combination *Limacinia moniliforma* (Fraser) comb. nov. is proposed for the fungus occurring on

Doryphora, *Epacris*, *Backhousia*, *Callistemon*, *Weinmannia*, *Eugenia*, *Ficus*, *Alyxia*, *Cryptocarya*, *Tristania*, *Polystichum*, and *Podocarpus* in Australia and on *Arctostaphylos* in California.

This species is readily distinguished from the preceding one by the more massive subiculum composed of hyphae whose cells are larger, nearly globose, and deeply constricted at the septa (Fig. 20), and by the apical appendages which form an erect tuft on the ascostroma (Figs. 18, 19).

Limacinia moniliforma (Fraser) Barr var. ***quinqueseptata*** var. nov. (Figs. 23–26)

A specie differt ad subiculum parvis, cobscure fusciscrinibus; ascostromata propius globosa, 145–205μ in altitudine ad 120–170μ in diametro, appendiculis crinitis levibus apicatis, 13–16μ in diametro, tenus 40μ per longitudinem; asci latiores, 80–96 × 25.5–40μ; sporidia longiora, plus brunnea, 5 septata, curva, 32–42 × 9.5–13μ.

Habitatio in ramis *Pseudotsugae taxifoliae* in British Columbia, Canada.

Limacinia moniliforma (Fraser) Barr var. ***quinqueseptata*** var. nov. (Figs. 23–26)

Differing from the species in subiculum forming small dull brown tufts; ascostromata more nearly globose, 145–205μ in height by 120–170μ in diameter, with a tuft of smooth-walled apical appendages, 13–16μ in diameter, up to 40μ in length; asci broader, 80–96 × 25.5–40μ; spores longer, dark brown, 5-septate, slightly curved, 32–42 × 9.5–13μ.

Habitat on twigs of *Pseudotsuga taxifolia*, British Columbia, Canada.

Specimen examined: BRITISH COLUMBIA, Canada: On *Pseudotsuga taxifolia*—Lake Cowichan, V.I., Sept. 10, 1943, R. E. Foster, DAOM 46165, TYPE.

This fungus, which was found in small amounts on twigs which also bore *Limacinia alaskensis*, bears a striking resemblance to *L. moniliforma*. The nature of the hyphae, the apical tuft of appendages on the ovoid to globose ascostromata (Figs. 23, 24), and the form of asci and spores (Figs. 25, 26) are all reminiscent of that species. The somewhat wider ascostromata and asci and the longer, 5-septate spores (Fig. 26) do not warrant specific separation of the two forms. For that reason it is proposed that this fungus be considered a variety of *L. moniliforma*, and be given the name *Limacinia moniliforma* var. *quinqueseptata* var. nov., thus stressing its most conspicuous distinguishing feature.

Limacinia multiseptata sp. nov. (Figs. 27–32)

Subiculum superficiale, in molibus laxis, crassis, atris, saepe conspicuum, in longitudine 1–2 cm., in latitudine 1–2 cm., in altitudine circiter 5 mm., ex dematiis fuscis hyphis, nimis ramosis, ex cellis torulosis circumdatis compositum, cellis variabilibus, maturis 40–50μ in diametris, vel 64μ crassis usque ad 50μ longis, muris levibus, cellis parvis 9.5–11μ crassis ad 8–11μ longis, flavis–fuscis, muris asperis. Ascostromata in mole densa locata,

globosa vel deplanata, (80)160–275(340)μ in diametro, atra muris tenuibus, composita ex uno aut duobus allis polygoniis et fuscis, aspera extra in inferiore parte plerumque hyphis, constantia ex brevibus (2–4) aut longis catenis, ramosis et inter se conjunctis aut cum subiculo, cum cellis in septis constrictis, ferme globosis usque ad 16–19μ in diametro; muris asperis, apicibus planis compositis ex variis cellis, apertis et lysigenosis. Asci fasciculati cum stipitibus brevis, clavati, duo vel octospori, muris ad apicem crassis, aparaphysati, 128–192 × 32–45μ. Sporidia multiseriata, elongato-fusiformia, recta vel curvata, primo hyalina, mox flava, ad maturitatem fusca, 13- vel 20-septata, 88–160 × 9.5–14.5μ.

Habitatio in ramis *Arctostaphylos sensitivae* et in cortice *Libocedri decurrentis* in California, United States of America.

Limacinia multiseptata sp. nov. (Figs. 27–32)

Subiculum superficial, dull black, forming massive cushions on twigs and bark, 1–2 cm. in length by 1–2 cm. in width by 5 mm. or more in height; composed of much-branched hyphae whose cells are deeply constricted at the septa, smooth or rough walled, bright yellowish to dark brown; cells variable in size, the oldest reaching a diameter of 40–50μ or 64μ in width by 50μ in length and smooth walled and dark brown, the younger 9.5–11μ in width by 8–11μ in length, yellow brown, and rough walled. Ascostromata seated on and partially immersed in the subiculum, globose to slightly depressed, (80)160–275(340)μ in diameter, black, thin walled; wall of one to two layers of polygonal brown cells, externally roughened in the lower two-thirds by hyphal appendages, formed of short (two to four cells) or longer chains which may branch and anastomose with one another or with cells of the subiculoid hyphae, cells constricted at the septa, nearly globose, up to 16–19μ in diameter, rough walled, yellow brown; apical region plane, formed of differentiated cells, opening by a lysigenous pore. Asci in a fascicle, long

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FIGS. 18 to 22. *Limacinia moniliforma.*

FIG. 18. Habit of ascostroma. FIG. 19. Hyphal appendage. FIG. 20. Portion of hypha from subiculum. FIG. 21. Ascus. FIG. 22. Ascospores.

FIGS. 23 to 26. *Limacinia moniliforma* var. *quinqueseptata.*

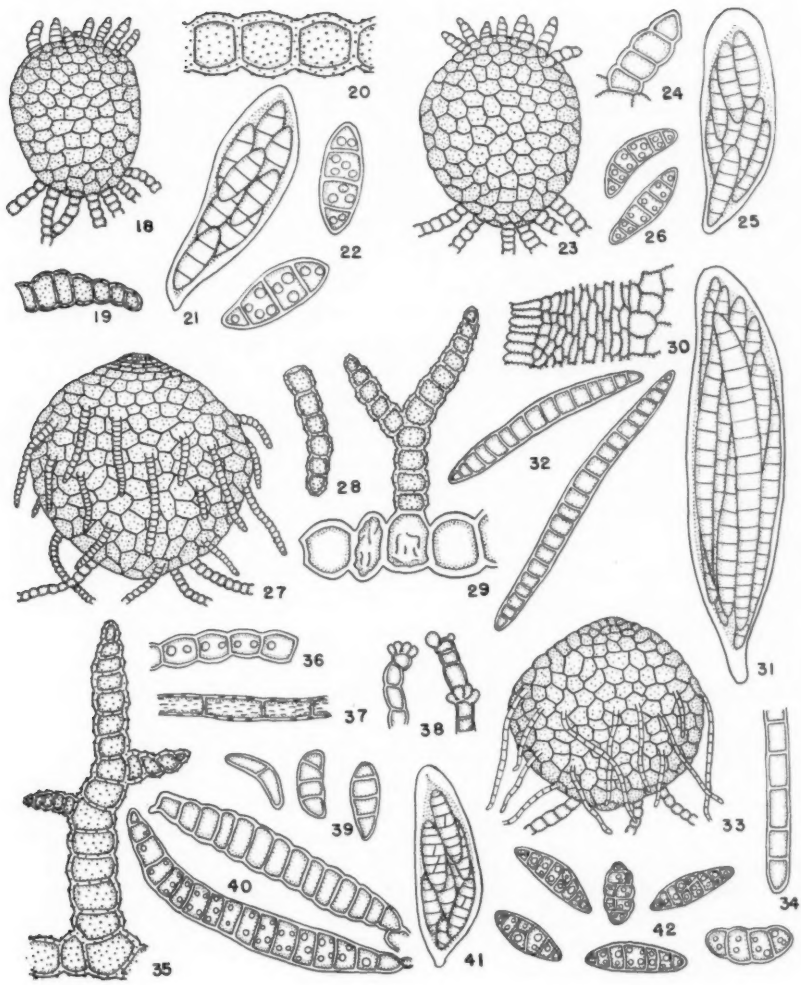
FIG. 23. Habit of ascostroma. Fig. 24. Hyphal appendage. FIG. 25. Ascus. FIG. 26. Ascospores.

FIGS. 27 to 32. *Limacinia multiseptata.*

FIG. 27. Habit of ascostroma. FIG. 28. Hyphal appendage. FIG. 29. Branch of subiculoid hypha. FIG. 30. Portion of apical region of ascostroma. FIG. 31. Ascus. FIG. 32. Ascospores.

FIGS. 33 to 42. *Capnodium spongiosum.*

FIG. 33. Habit of ascostroma. FIG. 34. Hyphal appendage. FIG. 35. Branching hypha of subiculum. FIG. 36. Second type of hypha. FIG. 37. Hypha from collection on *Pseudotsuga*. FIG. 38. Phialides on hyphal branches. FIG. 39. Conidia of *Arthrobotryum spongiosum*. FIG 40. Conidia of *Hyphosoma* type. FIG. 41. Ascus. FIG. 42. Ascospores. FIGS. 18, 23, 27, and 33. ×165. FIG. 22. ×630. The remainder ×420.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and broad clavate, narrowed below into a short, thick stalk, broadly rounded and thickened at the apex, bitunicate, (2-) 8-spored, aparaphysate, 128–192 × 32–45μ. Spores multiseriate in the ascus, elongate fusiform, ends bluntly rounded, straight, or curved, hyaline at first, soon becoming colored, and at maturity yellowish to dark brown, 13- to 20-septate, 88–160 × 9.5–14.5μ.

Habitat on branches of Manzanita, *Arctostaphylos sensitiva*, and on bark of *Libocedrus decurrens*, California, United States of America.

Specimens examined: CALIFORNIA, U.S.A.: On Manzanita—Zig Zag Fire trail, Mt. Tamalpais, Marin Co., March 21, 1926, H. E. Parks, TYPE, UC 276037 as *Aithalomyces rhododendri* Woronichin. On *Arctostaphylos sensitiva*—Mt. Tamalpais, Marin Co., March, 1933, V. P. Mentzer, California Fungi 301, UC 498813 as *Aithalomyces rhododendri* Woronichin. Cascade Trail above Mill Valley, Mt. Tamalpais, Marin Co., Aug. 23, 1942, W. B. Cooke 16789, UC 681322 as *Aithalomyces rhododendri* Woronichin. On bark of *Libocedrus decurrens*—Smith River, Del Norte Co., July 13, 1933, L. Bonar, UC 960424 as *Arthrobotryum spongiosum* Hoerl.

This species is readily distinguished from all others of the genus by the long fusiform, multiseptate spores (Figs. 31, 32) for which it is named. Most of the species of *Limacinia* have spores with three to five transverse septa, although *L. mucronata* (Mont.) Sacc. has 6- to 7-septate spores. Spegazzini (in Saccardo (26)) proposed the name *Ophiocapnodium* for a fungus which had elongate, multiseptate spores. This genus is further distinguished, however, by the elongate, cylindrical beak of the perithecium, a character which is certainly not present in *L. multiseptata*. Species with elongate, multiseptate spores have been included in genera whose other members have few-septate spores. The genus *Leptosphaeria* may be cited as a precedent for the present inclusion of a species with such divergent spores in the genus *Limacinia*.

The appendages of the ascostromata of *L. multiseptata* are quite distinctive. These may be short and composed of only three or four cells or much longer (Figs. 27, 28), in all cases are much constricted at the septa, arise from the sides of the wall, and are pendent, frequently anastomosing with the hyphae of the subiculum (Fig. 29). They appear to be intermediate between those of *L. alaskensis* which arise from the sides of the wall, are only slightly constricted at the septa, and are long and pendent, and those of *L. moniliforma* and its variety which are constricted at the septa and form an erect tuft at the apex of the ascostroma.

The apical region in this fungus shows more differentiation of cells than does that of any other here discussed. The lysigenous pore is surrounded by a row of vertically elongated, narrow cells. Beyond this are several rows of rather small, narrow, horizontally elongated cells which merge with the larger polygonal cells of the side wall (Fig. 30). Before maturity, the apical region

is filled with hyaline, thin-walled cells which are readily ruptured by the maturing asci as they push upward. The elongate layer of cells previously referred to is forced upward and forms a short collar on mature ascostromata.

Capnodium Mont.

As in the case of *Limacinia*, numerous genera have been based on variable characters in the group of sooty molds which have brown, muriform ascospores. A single species may show a wide range in shape of ascostromata, presence or absence of appendages, etc. The earlier interpretation of *Capnodium* as having stalked fruiting bodies has been shown by Miss Fisher (7) to be untrue. The single species with muriform spores which was studied is here included in the genus *Capnodium*.

Capnodium spongiosum sp. nov. (Figs. 33–42)

Subiculum superficiale, in molibus laxis, crassis, atris, saepe conspicuum, plerumque ex dematiis fuscis hyphis, nimis ramosis, ex cellis torulosis compositum, cellis variabilibus, 16–44μ in diametris, muris asperis, minor ex dematiis fuscioribus hyphis, ramosis, ex cellis longiore compositum, cellis 13–16μ longis ad 9.5–13μ crassis, muris levibus. Ascostromata in mole densa locata, atra, globosa, 160–240(340)μ in diametro; muris tenuis, composita ex duobus aut tribus allis polygoniis et fuscis; in apicibus partis apertis ad foramenis; appendiculis fuscis, septatis, simplicis vel ramosis, levis vel asperis, hyphoideis. Asci fasciculati cum stipitibus brevis, obclavati, ventricosi, muris ad apicem crassis, aparaphysati, octospori, 64–100 × 22.5–40μ. Sporidia conglobata, ellipsoidea–fusoidea, forma inconstantissima, uno lata plana vel curva, flava vel fusca, 3- vel 7-septata, septa longitudinaliter in media cellula, non vel graciles constricta in septata, guttulata in cellula, 24–51 × (8-)9.5–13μ.

Habitatio in cortice *Libocedri decurrentis*, *Cupressi sargentii*, *Chamaecyparis lawsonianae*, et *Pseudotsugae taxifoliae* var. *glaucae*, California et Washington, United States of America.

Capnodium spongiosum sp. nov. (Figs. 33–42)

Subiculum superficial, dull black, massive, forming cushions on the bark, composed of two types of hyphae: the greater portion of the cushion consisting of much branched hyphae whose cells are deeply constricted at the septa and more or less globose, 16–44μ in diameter, rough walled, yellowish to dark brown; intermingled hyphae which are less constricted at the septa, cells somewhat elongated, 13–24μ in length by 9.5–13μ in width, dark brown, smooth walled or roughened by longitudinal striations, containing one or more guttules. Ascostromata black, seated on and in the subiculum, covered with numerous long, narrow, scarcely constricted, dull brown, smooth or finely roughened, occasionally branched hyphal appendages, whose cells are 16–32μ in length by 8–9.5μ in width; thin walled, of two to three layers of polygonal,

brown cells, with a pore at the apex at maturity but lacking any ostiolar differentiation of cells, more or less globose, 160–240(340)μ in diameter. Asci in a fascicle, obclavate, broadened in the lower half, narrowed abruptly to a short, thick, knob-like stalk or rounded and sessile, bitunicate, thickened at the broadly rounded apex, 8-spored, aparaphysate, 64–100 × 22.4–40μ. Spores irregularly crowded in the ascus, ellipsoid–fusoid, variable in shape, one side flattened or slightly curved, ends broadly rounded, light yellowish to dark brown, very variable in septation, 3- to 7-septate, with a longitudinal septum in one or two of the central cells of most of the 5- and 7-septate spores, not or somewhat constricted at the septa, one or more globules in each cell, 24–51 × (8-)9.5–13μ.

Habitat on bark of *Libocedrus decurrens*, *Cupressus sargentii*, *Chamaecyparis lawsoniana*, and *Pseudotsuga taxifolia* var. *glauca* in California and Washington, United States of America.

Specimens examined: CALIFORNIA, U.S.A.: On *Libocedrus decurrens*—above 18 Mile Creek, Smith River, Del Norte Co., April, 1937, November, 1938 (two dates on label), H. E. Parks, California Fungi 418, TYPE, UC 681382, as *Arthrobotryum spongiosum* Hoerl. Smith River, Del Norte Co., May, 1937, H. E. Parks, 5936, UC 692997. On *Cupressus sargentii*—two miles east of Camp Meeker, Sonoma Co., Sept. 28, 1947, M. A. Nobs and A. R. Kruckeberg, UC 960406. On *Chamaecyparis lawsoniana*—Rock Creek Lodge, south fork of the Smith River, Del Norte Co., Aug. 16, 1949, W. B. and V. G. Cooke 25536, Mycobiota of North America 400, UC 915700.

WASHINGTON, U.S.A.: On *Pseudotsuga taxifolia* var. *glauca*—one mile south of Springdale, Stevens Co., April 26, 1947, W. B. Cooke 19660, WS 38815, paratype of *Helminthosporium pseudotsugae* W. B. Cooke.

The first four collections cited were determined as *Arthrobotryum spongiosum* Hoerl. Synnemata of this species were found in two of the collections. These produced 3-septate, brown conidia (Fig. 39), measuring 29–40 × 11–13μ, and agree with Miss Hoerl's (11) description of that species. They may possibly be connected in the life cycle of the *Capnodium*. Further evidence is necessary, however, before a definite statement could be made to that effect. The collection on *Pseudotsuga* is identical with the others with the exception of the hyphae of the subiculum. The subiculum does not contain hyphae of the first type (Fig. 35), and those referable to the second type (Fig.36) have the walls roughened by longitudinal striations and are more distantly septate (Fig. 37).

Conidia found in these collections are similar to those found associated with *Limacinia alaskensis* and are referred to the genus *Hyphosoma* (Fig. 40). They arise from subiculoid hyphae of the second type, are cut off from a short branch, and are elongate–fusoid, straight or curved, tapering at the ends, smooth and thick walled, dark brown, with clear cellular contents or containing several small guttules, 9–17(18)-septate, 74–160 × 13–17.5μ. No definite connection of these conidia with the perfect stage has been established.

In addition, phialides similar to those found in collections of *Limacinia alaskensis* occur on short side branches arising from hyphae of the second type. These occasionally show proliferation of the side branch so that the first group of globose cells forms a ring around the branch which is topped by a second ring of cells, shown in Fig. 38.

Miss Fraser (10) emended the description and figured *Capnodium mucronatum* Mont. from several hosts in New South Wales. This species is certainly closely related to *C. spongiosum*. It too possesses long fusiform conidia, phialides, ascostromata with hyphal appendages (as in Figs. 33, 34), and similarly shaped asci (Fig. 41) and ascospores (Fig. 42). However, the measurements of *C. mucronatum* are larger in all respects than those of *C. spongiosum*. The ascospores, in particular, serve to distinguish the two species. Miss Fisher described those of *C. mucronatum* as fusiform, greenish-brown, end cells thinner and paler at the apices, which at maturity are slightly papillate, transversely 7-septate, and measure 50–70 × 15–18μ. The ascospores of *C. spongiosum* are smaller, more deeply colored, and lack the mucronate tips on the spores (Fig. 42). They are also muriformly septate, whereas the spores of *C. mucronatum* appear to lack the longitudinal septum, which caused Saccardo (27) to place this species in *Limacinia*.

From the description of *Coccodinium laricis* W. B. Cooke and Shaw (5), that species could be identical with the fungus under discussion. Type material was obtained from Dr. C. G. Shaw (WS 4862), but proved to be too sparse to allow intensive study. The ascospores, however, were distinctly shorter and narrower than those of the species of *Capnodium* and invariably bore a longitudinal septum which in most cases extended throughout the spore except for the tip cells. It was concluded that this species was distinct from the *Capnodium* here designated as *C. spongiosum*. This name was suggested by Dr. S. J. Hughes, who has examined two of the collections cited here.

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(i) ***General***

Manuscripts, in English or French, should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

Manuscripts and illustrations should be carefully checked before they are submitted. Authors will be charged for unnecessary deviations from the usual format and for changes made in the proof that are considered excessive or unnecessary.

(ii) ***Abstract***

An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

(iii) ***References***

References should be listed **alphabetically by authors' names,** numbered, and typed after the text. The form of the citations should be that used in this journal; in references to papers in periodicals, titles should be given and inclusive page numbers are required. All citations should be checked with the original articles, and each one referred to in the text by the key number.

(iv) ***Tables***

Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Vertical rules should be used only when they are essential. Numerous small tables should be avoided.

Illustrations

(i) ***General***

All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic numerals, and each figure should be referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left-hand corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations (see Manuscripts (i)).

(ii) ***Line drawings***

Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used unless it is desired to have all the co-ordinate lines show. All lines should be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots should be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm. high when reproduced in a cut 3 in. wide.

Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. In large drawings or groups of drawings the ratio of height to width should conform to that of a journal page but the height should be adjusted to make allowance for the caption.

The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.

(iii) ***Photographs***

Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard with no space or only a **very** small space (less than 1 mm.) between them. In mounting, full use of the space available should be made (to reduce the number of cuts required) and the ratio of height to width should correspond to that of a journal page (4¾ × 7½ in.); however, allowance must be made for the captions. Photographs or groups of photographs should not be more than 2 or 3 times the size of the desired reproduction.

Photographs are to be submitted in duplicate; if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

Reprints

A total of 50 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½ × 11 in.) and including the space occupied by illustrations. An additional charge is made for illustrations that appear as coated inserts. The cost per page is given on the reprint requisition which accompanies the galley.

Any reprints required in addition to those requested on the author's reprint requisition form must be ordered officially as soon as the paper has been accepted for publication.

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